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Via electronic mail

December 17, 2009

Docket ID No. EPA-HQ-ORD-2009-0217
via email to ORD.Docket @epa.gov

RE: Docket ID No. EPA-HQ-ORD-2009-0217
Draft Toxicological Review of Chloroprene (September 2009)
Comments on Behalf of DuPont Performance Elastomers

Dear Docket Control Officer:

On behalf of DuPont Performance Elastomers (DPE), I am pleased to submit comments prepared both by and for DPE on the Draft Toxicological Review of Chloroprene.

Per letter dated November 25, 2009 from EPA (Dr. Abdel Kadry) to DuPont (Andrea Malinowski), EPA has advised that comments submitted by December 17th will be provided to the Peer Review Panel members two weeks before the meeting. The Peer Review Panel meeting is scheduled for January 6, 2009. It is respectfully requested that the Peer Review Panel members be provided with the attached comments as soon as practicable due to the upcoming holidays, but no later than December 23rd.

DPE also contacted Versar to offer courtesy paper copies of the comments. Versar indicated it would be helpful to receive them and that six (6) copies would be required. Accordingly, to aid in timely distribution of the comments to the Peer Review Panel and as a courtesy, six (6) paper copies and six (6) text-searchable CDs of DPE's comments are being provided today by overnight mail to Versar. Additional copies will be provided to Versar, upon request, if more are needed.

Finally, if EPA desires paper copies or CDs of the comments, please contact me directly by phone or e-mail and we will promptly handle the request.

Very Truly Yours,

Andrea V. Malinowski

Attachment: Comments on the September 29, 2009 Draft of the IRIS Toxicological Review for Chloroprene - *Submitted on Behalf of DuPont Performance Elastomers*

CC: 6 paper copies and 6 CDs by Overnight Mail to
Versar, Inc. Attn: Mr. David Bottimore
6850 Versar Center
Springfield, Virginia 22151

Comments on the September 29, 2009 Draft of the IRIS Toxicological Review for Chloroprene

Submitted on Behalf of DuPont Performance Elastomers

By

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Submitted to:
U.S. Environmental Protection Agency
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Washington, DC 20460

December 17, 2009

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Comments on the September 29, 2009 Draft of the IRIS Toxicological Review for Chloroprene

DuPont Performance Elastomers (DPE), the only domestic producer of β-chloroprene monomer (chloroprene), appreciates this opportunity to comment on the September 29, 2009 Draft of the IRIS Toxicological Review for Chloroprene. DPE, in conjunction with DuPont Haskell Global Centers and The International Institute of Synthetic Rubber Producers Chloroprene Scientific Oversight Committee (IISRP-SOC) are committed to the development and conduct of research critical to understanding the potential health risks from exposure to chloroprene.

With the assistance of the reviewers listed on the cover page of this submission, DPE has prepared comments on the September 29, 2009 Draft of the IRIS Toxicological Review for Chloroprene. These comments are focused on those areas that we respectively request be reexamined and clarified before the IRIS Review is finalized. We have outlined comments, both qualitative and quantitative, that will better inform the conclusions drawn by the USEPA.

Specifically, we intend to comment on key arguments cited by the USEPA in the Draft Review, notably:

> *"…evidence of an association between liver cancer and occupational exposure to chloroprene; 2) some evidence of an association between lung cancer risk and occupational exposure; 3) the proposed mutagenic mode of action; and 4) structural similarities between chloroprene and known human carcinogens, butadiene and vinyl chloride."*

We also provide relevant new data on toxicokinetics and mode of action (MOA) that pertain to the critical review of the available epidemiological, toxicological, and mechanistic data for chloroprene, and how those data are used in the derivation of the proposed Reference Concentration (RfC) and Unit Risk (UR). An overview (Executive Summary) of these comments is provided, with more detailed comments in the main sections and supporting attachments that follow.

Overview of Comments/Executive Summary

On behalf of DuPont Performance Elastomers (DPE), scientists considered to be experts in their respective fields of epidemiology, mutagenicity/genotoxicity, toxicokinetics, and dose response/cancer risk assessment have conducted a critical review of the document entitled, "Draft of the IRIS Toxicological Review for Chloroprene, September 29, 2009" (herein referred to as the "Draft Review"). This comment document (herein referred to as "Comments") has been prepared for submission to Integrated Risk Information System (IRIS) Program of the U. S. Environmental Protection Agency (USEPA), as well as the external peer review panel selected for the Draft Review.

After careful review, we respectfully disagree with certain approaches and conclusions described in the Draft Review. Our comments will focus on five major technical issues that warrant consideration and resolution by the USEPA prior to finalization of the Draft Review. Further, there are five new studies, two which have recently been submitted to the USEPA, two in final stages of report preparation that will be finalized in January, and the last on *in vivo* physiologically based toxicokinetic (PBTK) modeling that is expected to be available in February, 2010, which collectively contain critical information on mode of action and toxicokinetics that will affect USEPA's quantitative risk assessment. Comprehensive details associated with each of these issues are provided in the main sections of this Comment document. In addition, comments are annotated if a specific issue is related to a particular Charge Question to the external peer review panel. In summary, the major technical issues include:

1. Interpretation of the Epidemiological Studies (General Charge Question 1, Chemical-Specific Question C.1)

The Draft Review provides a summary of epidemiological studies of individuals working in chloroprene manufacturing facilities in several countries. We contend that the conclusions reached by the USEPA as to the carcinogenicity classification (e.g., likely to be carcinogenic to humans) of chloroprene in humans are not supported by the data for the following reasons:

- **The Draft Review did not follow the USEPA approved method to assess epidemiological data quality, as detailed in the guidelines for the assessment of human cancer risk (USEPA 2005) (Section 1.A).**
    - The Draft Review did not apply nor consider the ten-point evaluation criteria specified by the USEPA in their *Guidelines for Carcinogen Risk Assessment* (USEPA 2005).
    - Consequently, the Draft Review did not assign a study-specific weight to each study cohort to reflect the quality of the study with regard to the relative strengths and limitations of each study and the validity of the conclusions that could be drawn.
    - The Draft Review actually gave more weight to inferior studies in non-US worker populations and to two preliminary studies in US workers that were superseded by the more comprehensive Marsh *et al*. (2007a, b) study of that same population.

- By giving limited consideration to the Marsh *et al*. (2007a, b) study, erroneous conclusions were reached regarding the weight-of-evidence for the association between chloroprene and cancer mortality.

- **One of the key studies cited by USEPA as the basis for linking chloroprene exposure with cancer (Leet and Selevan, 1982) was superseded by the Marsh *et al*. (2007a, b) study. The Marsh *et al*. study of cohorts in the United States, Ireland and France did not report an association between exposure to chloroprene and the incidence of either total cancers or cancers of the lung or liver (Section 1.D).**
  - The Marsh *et al*. (2007a, b) study is the largest and most comprehensive study that has been published, providing the most complete documentation of exposure.
  - Weight-of-evidence assessments in the peer-reviewed literature (Bukowski 2009) concluded that the most reliable information regarding the potential for lung and liver cancer from chloroprene exposure is provided by Marsh *et al*. (2007a, b).
  - The Pell (1978) and Leet and Selevan (1982) studies are early reports of mortality for the Louisville, Kentucky cohort. The Marsh *et al*. (2007a, b) study extends follow-up for this cohort prior to and after these two reports. Therefore, the Pell (1978) and Leet and Selevan (1982) studies do not include results independent from those reported by Marsh *et al*. (2007a, b).

- **Interpretations of the Chinese, Russian, and Armenian cohorts (Li *et al*. 1989; Bulbulyan *et al*. 1998, 1999) failed to acknowledge the imprecise and unstable estimates of mortality and incidence ratios due to very low expected counts for liver and lung cancer mortality (Section 1.B).**
  - The reported expected counts for liver and lung cancer mortality and incidence are very low for the Chinese, Russian, and Armenian cohorts (Li *et al*. 1989; Bulbulyan *et al*. 1998, 1999), with many of the expected counts below 1.0 and most below 2.0. These low expected values indicate either inaccurately applied population reference rates or a questionable approach to estimating expected deaths using the selected population mortality rates.
  - Random chance may influence the occurrence of a limited number of observed outcomes that may overwhelm the very small estimate of expected health outcomes in a ratio measure.
  - A cautious approach should be considered when interpreting the excess risk estimates associated with these very low expected counts, especially when liver cancer is common in these populations.

- **In addition, the Chinese, Russian, and Armenian studies have limitations and confounders that limit the interpretation and conclusions of their reported findings (Section 1.A.2).**
  - The methodological limitations of the Chinese, Russian and Armenian epidemiological studies (Li *et al*. 1989; Bulbulyan *et al*. 1998, 1999) were not adequately considered in the Draft Review in accordance with USEPA criteria (USEPA 2005) for epidemiological data quality.
  - Unlike the cohorts evaluated by Marsh *et al*. (2007a, b), processing differences may result in higher exposure to vinyl chloride, which confounds interpretation of the results with regard to chloroprene exposure.

- Since the identification of limitations in these studies, there has been no attempt to update the Chinese, Russian and Armenian cohorts to confirm the preliminary claims of an association between exposure to chloroprene and cancer mortality. Only the Marsh *et al*. (2007a, b) study has been conducted to update earlier studies of mortality among chloroprene exposed workers (Pell 1978; Leet and Selevan 1982).

Taken together, the referenced epidemiologic studies in the Draft Review do not establish a clear causal inference of liver and lung cancers due to occupational chloroprene exposure. Consequently, one of the USEPA's arguments to justify a proposed "likely to be carcinogenic to humans" classification for chloroprene is not supported by a revised assessment of the epidemiological data.

2. Interpretation of the Mode of Action Based on the Mutagenicity and Genotoxicity Data (General Charge Questions 1, 2, Chemical-Specific Question C.3)

A critical evaluation of the cytotoxic and genotoxic profiles of chloroprene suggests that this chemical involves key events in the MOA that differ from the carcinogens 1,3-butadiene and isoprene. Chloroprene's genotoxicity profile lacks several attributes that would provide the necessary support for a mutagenic MOA. Furthermore, EPA did not evaluate possible alternative MOA in the Draft Review.

- **Standard *in vivo* tests for genotoxicity are negative (Section 2.A).**
  - Chloroprene, unlike butadiene and isoprene, does not exert genetic toxicity to somatic cells *in vivo.*
  - In addition, neither chloroprene nor its major epoxide metabolite was genotoxic in mammalian cells treated *in vitro*.
  - In order to have confidence in a mutagenic MOA, one would expect the candidate chemical to produce evidence of genotoxicity in mammalian somatic tissue(s) of the species in which it induces tumors.
  - Studies suggest that the epoxide metabolite of chloroprene is effectively detoxified during *in vivo* exposure conditions.
- **Lack of consistent data for point mutation induction (Section 2.A.3, 2.A.4)**.
  - The ability of chloroprene to produce point mutations *in vitro* (bacteria) is equivocal, at best. Chloroprene did not induce mutation in cultured mammalian cells.
  - The conflicting specificities between *in vitro* point mutation and DNA adduct induction and *in vivo ras* mutations found in target site tumors may be of an origin other than chloroprene-induced. The *ras* "mutagen finger print" of A to T specificity needs to be reconciled with the *in vitro* mutation and DNA adduct data, which clearly show a G-to-C transition profile, in order to fully support a mutagenic MOA. The inconsistency in specificity raises questions as to whether the A to T mutations are a reliable indication of *in vivo* point mutation induction or are the consequence of other secondary processes.

- **A non-genotoxic MOA for chloroprene should be considered (Section 2.B)**.
    - Some evidence exists to suggest that the reported target-site specific toxicity of chloroprene in mice could be attributed to localized cytotoxicity with subsequent induction of hyperplasia and cell regeneration followed by promotion of pre-existing proto-oncogene mutations.
    - Aspects of this alternative MOA fits the known toxic and DNA reactivity attributes of chloroprene. Butadiene and isoprene, used as comparison carcinogens, are characterized by different toxicity, genotoxicity and oncogene mutation profiles.

Chloroprene metabolites and/or oxidative degradation products appear to be DNA reactive *in vitro*; however, extrapolation of that information directly to a mutagenic MOA is not compelling and a non-mutagenic MOA based on target site cytotoxicity should be considered.

3. Consideration of Species Differences in Toxicokinetics and Target Tissue Dosimetry (General Charge Questions 1, 2)

In the Draft Review, a brief summary is provided for several studies that demonstrate, both qualitatively and quantitatively, differences across species in the toxicokinetics of chloroprene (Munter *et al*. 2007a, b, 2003; Himmelstein *et al*. 2004a, 2001a, b; Cottrell *et al*. 2001; Summer and Greim 1980; Hurst and Ali 2007). Consideration of these differences is critical in the determination of potential human health risk effects following exposure to chloroprene for the following reasons:

- **Significant species differences in metabolism are documented (Section 3.A).**
    - It is clear from the peer-reviewed literature (Munter *et al*. 2007a, b; Himmelstein *et al*. 2004a, Cottrell *et al*. 2001) that there are significant differences in metabolism of chloroprene across species that can impact target tissue dose.
    - Because the MOA proposed for chloroprene in the Draft Review may be dependent on the generation of a metabolite in the target tissue, it is important that the determination of Human Equivalent Concentrations (HECs) incorporate species differences in metabolism.
- **Previous analyses support the use of the physiologically based toxicokinetic (PBTK) model (Section 3.B).**
    - Using a peer-reviewed PBTK model (Himmelstein *et al*. 2004b), internal dose for the lung was determined and applied in a dose-response analysis of lung tumors. A better correlation was found between the incidence of lung tumors and internal dose, compared to that seen when the external exposure concentration values were used as the "dose", supporting an association between the target tissue dose estimated by the model and the observation of lung tumors in mice and rats.
- **New Data support the use of the PBTK model (Section 3.B).**
    - The available peer-reviewed PBTK model (Himmelstein *et al*. 2004b) was not incorporated in the dose-response modeling for chloroprene in the Draft Review due to the lack of time-course data for chloroprene in the blood.
    - Data now exists (Attachment B) to support the application of this quantitative method allowing for the incorporation of the PBTK model in the determination of

HECs. This information is being prepared into a report which is expected to be available in February, 2010.

- **Analyses from additional studies are available that will increase confidence in the PBTK model parameters (Section 3.B).**
    - Additional studies are near completion and provide new refined parameters of *in vitro* chloroprene metabolism in liver and lung microsomes of female mice and rats, in kidney microsomes of male and female mice and rats, and mixed-gender pooled kidney microsomes from humans. The data have been analyzed using a probability analysis completed as a key step to better define parameter variability when scaling the parameters for incorporation in the *in vivo* PBTK model. This information is being prepared into a report (IISRP-17520-1388) which will be available to USEPA by the end of January 2010.
    - The use of the refined metabolic parameters determined as a result of this study will increase confidence and decrease uncertainty in the parameters applied and therefore in the PBTK model simulations used in dosimetric adjustments for human exposures (IISRP-17520-1389).
- **New genomics information provides evidence of differences in response across species (mice and rats) that reflects more than just kinetic differences in the production and retention of reactive metabolites (Section 3.C).**
    - The gene expression changes observed in the rat in a recently completed genomics study (IISRP-12828-1389) occurred at comparatively higher chloroprene exposure concentrations than those observed in the mouse. When exposure was normalized to preliminary measures of internal dose based on the PBTK model, the internal doses in the rat were highly consistent with those observed in the mouse. These results lend support to the hypothesis that the observed differences in tumor response in the chronic animal bioassays are related to species-specific differences in metabolism. The genomic report (IISRP-12828-1389) will be finalized before the end of January 2010.

Now that a refined, validated PBTK model is near completion, the use of tissue-specific dosimetry to derive the HEC is justified for use in the quantitative dose-response analysis of the rodent bioassay data. The application of the *in vivo* PBTK model to probabilistic dose response modeling (IISRP-17520-1389) is expected to be available in February, 2010.

4. USEPA decision points in the determination of the UR (Chemical-Specific Questions C.2, C.4, C.5).

In the Draft Review, the results of the National Toxicology Program (NTP 1998) two-year bioassay in rats and mice have been relied upon in the determination of the potential human health effects for chloroprene. In the absence of positive epidemiological studies, these data must be interpreted with caution and several decision points reconsidered prior to finalizing the Draft Review. These include:

- **In the determination of the Unit Risk (UR) for carcinogenicity, URs from multiple tumor types should not be summed (Section 4.B).**
    - In the Draft Review, multiple tumor types are modeled and the URs summed to determine the final UR. This summation is conducted under the assumption of statistical independence. However, because the MOA for the development of all tumors is proposed in the Draft Review to be the same for all tumors and dependent upon the generation of the same metabolites, mechanistic or biological independence is not established. Therefore, the summing of URs is overstating the potential carcinogenicity of chloroprene.
- **The most appropriate approach for derivation of the UR for chloroprene if animal data are used is to rely upon the most sensitive tumor endpoint (i.e., lung tumors) in the most sensitive species (Section 4.B).**
    - By applying the standard USEPA approach of relying upon the most sensitive response in the most sensitive species, in combination with the application of a time-to-tumor model that quantitatively considers competing risks of death, the resulting UR should be protective of the other responses observed in the NTP (1998) study. This approach removes the need to sum URs, which is expected to overestimate the potential risk of effects from chloroprene exposure in the mouse.
    - Many of the assumptions in the method applied (e.g.., normality around the maximum likelihood estimate or MLE) are inappropriate.
- **Because the MOA proposed for chloroprene in the Draft Review is dependent upon target tissue dose, it is critical that the HEC values take into consideration important species differences in metabolism (Section 4.B).**
    - The *Guidelines for Carcinogen Risk Assessment* (USEPA 2005) indicate that the determination of target tissue dosimetry is best accomplished within the framework of a PBTK model.
    - A peer-reviewed PBTK model is available for chloroprene (Himmelstein *et al*., 2004b) and has now been refined and validated per recently collected data (Section 3) and should be applied. The application of this model is critical to the quantitative interpretation of the existing toxicological database for chloroprene and will put in perspective the non-positive epidemiological data with the positive rodent bioassay results by comparing responses across species at the target tissue level.

Based on these considerations, the approach to the development of the UR should be revised, focusing on the assessment of the most sensitive endpoint in the most sensitive species, incorporating the PBTK model.

5. USEPA's quality control in reporting of chloroprene data (General Charge Question 1)

In comparing information provided in the Draft Review to that in the primary literature, numerous inconsistencies were noted (Section 5). In addition, information on the production of chloroprene noted in the Draft Review is not current and there are issues in attempting to

duplicate some of the quantitative analyses. We respectively request that these items (Attachment C of this comment report) be addressed prior to finalization of the Draft Review.

Summary and Conclusions

There are several major issues in the interpretation of the available toxicological data for chloroprene in the Draft Review that warrant change. With regard to the epidemiological data, there is no compelling evidence for an increased risk of mortality from total cancer or lung or liver cancer when the available studies are evaluated using a comprehensive weight-of-evidence approach. Although animal studies provide a positive response for carcinogenicity, species-specific differences in response to chloroprene exposure are observed. These likely reflect quantitative differences in toxicokinetics across species, specifically related to differences in both species differences in sensitivity and in metabolism and detoxification of potentially active metabolites. In the current Draft Review, no attempt was made to quantitatively account for these differences between the mouse, rat, and human. When genotoxicity/genomics, mode of action and toxicokinetic data are considered in an integrated manner, these data strongly suggest that the responses from chloroprene are largely confined or unique to the mouse. Because of these differences, use of the mouse data, in the absence of positive epidemiological data that can be used quantitatively, must incorporate tissue-specific dosimetry and metabolic differences.

Detailed Comments on the Current Draft Review

The scientists submitting these detailed comments respectfully request that these issues be considered by the USEPA and submitted to the external peer review panel before the Draft Review is finalized. These issues will impact both the qualitative and quantitative conclusions drawn by the USEPA as they relate to the potential human health effects of chloroprene.

1. Issue for Resolution: USEPA Interpretation of the Available Epidemiological Studies

The USEPA's Draft Review considered a number of occupational cohorts in several countries in their review of the potential carcinogenicity of chloroprene including: Pell (1978), Leet and Selevan (1982), Li *et al*. (1989), Bulbulyan *et al*. (1998, 1999), Colonna and Laydevant (2001), and Marsh *et al*. (2007a, b). These epidemiologic studies were published over a 30-year period, from 1978 to 2007. The specific health endpoints identified in the Draft Review are liver and lung cancers, principally mortality due to these cancers. The Draft Review concluded that chloroprene is "likely to be carcinogenic to humans" on the basis of "evidence of an association between liver cancer risk and occupational exposure to chloroprene" and "some evidence of an association between lung cancer risk and occupational exposure". As described in the following sections, when the quality (i.e., strengths and limitations) of the individual epidemiological studies are considered on a weight-of-evidence basis, these conclusions are not supported.

The following sections comprehensively address the accuracy, completeness and weighting of evidence associated with the USEPA review and interpretation of available epidemiological studies of working populations exposed to chloroprene. Our comments address the relative strengths and limitations of the published epidemiological studies of chloroprene-exposed populations included in the Draft Review, and analyze how these data were used to reach conclusions about potential health effects from chloroprene exposure. Each of our comments is supported by the relevant data from the epidemiological studies. We also provide other supporting data from the Marsh *et al*. (2007a, b) investigators, which was discussed but not provided in the published studies. In addition to the comments provided below, a detailed discussion is provided by the investigators of Marsh *et al*. (2007a, b) of the strengths and weaknesses of the four epidemiological studies that were given significant weight in the Draft Review in drawing conclusions related to the potential carcinogenicity of chloroprene in humans (see Attachment A). Many of the strengths and limitations of these studies also have been discussed in the peer-reviewed literature by Rice and Boffetta (2001), Acquavella and Leonard (2001), and Bukowski (2009).

1.A. The Draft Review did not assign a study-specific weight to each study cohort reflecting the relative strengths and limitations of the study but rather assigned relatively equal weight to all studies resulting in limited reliance upon the Marsh *et al*. (2007a, b) study. By not fully noting the relative strengths of the Marsh *et al.* (2007a, b) study, which provides the most reliable information regarding the potential for lung and liver cancer from chloroprene exposure, USEPA arrives at a conclusion not supported by the available evidence.

The USEPA has set forth criteria for the evaluation of epidemiological data in the *Guidelines for Carcinogen Risk Assessment* (USEPA 2005) and demonstrated by Bukowski (2009) for chloroprene. These criteria should have been applied in the Draft Review in the evaluation and determination of the weight-of-evidence available from each of the individual epidemiological studies for chloroprene. For the analysis of human tumor data, study quality and weight-of-evidence should be assessed using ten criteria established by the USEPA as those most appropriate for judging epidemiological studies used in risk assessment (USEPA, 2005), namely: (1) clear objectives; (2) proper selection and characterization of comparison groups (cohort and reference); (3) adequate characterization of exposure; (4) sufficient duration of follow-up; (5) valid ascertainment of cases of death or disease; (6) proper consideration of bias and confounding; (7) sample size; (8) clear, proper and well-documented methods for data collection and analysis; (9) adequate response (minimal loss to follow-up); and, (10) clear and well-documented results.

Bukowski (2009) evaluated the quality and weight-of-evidence associated with eight mortality or morbidity studies of seven chloroprene-exposed cohorts from six countries using these ten characteristics (**Table 1**). Studies were assigned to categories of high, medium or low quality for each of the ten USEPA quality criteria. Bukowski appraised the paired studies by Marsh *et al*. (2007a, b) as the highest quality studies to assess the potential for cancer effects among chloroprene exposed workers. Bukowski (2009) concluded that the four-cohort Marsh *et al*. (2007a, b) study was by far the most methodologically rigorous study conducted to date, having the most comprehensive exposure assessment and follow-up and detailed documentation. Specifically, the Marsh *et al*. study possessed the soundest methodological design, the largest overall cohort size, the highest *a priori* statistical power, the most comprehensive data collection for both cancer mortality ascertainment and follow-up time, and the most detailed assessment of exposure to chloroprene and associated agents (i.e., vinyl chloride).

Importantly, across the eight cohorts evaluated, the four cohorts from the Marsh *et al*. (2007a, b) study had the top overall quality rankings (accounting for all ten criteria) in the order: Louisville, Kentucky (L); Maydown, Northern Ireland (M); Ponchartrain, Louisiana (P); and Grenoble, France (G). The large Louisville, Kentucky cohort, which comprises 44% of the overall Marsh *et al*. (2007a, b) cohort, carried the greatest weight for purposes of risk assessment, ranking high for nine of the ten criteria and medium to high for one criterion.

In contrast, a review by Rice and Boffetta (2001) assessed studies of chloroprene-exposed cohorts published up to that time. The review comprised available epidemiological studies analyzing cohorts in the United States (Pell 1978), China (Li *et al*. 1989), Russia (Bulbulyan *et al*. 1998), and Armenia (Bulbulyan *et al*. 1999). The Rice and Boffetta (2001) review listed significant limitations of these studies including unclear documentation for cohort enumeration, inadequate reference rates for standardized ratios, a lack of detailed histopathology of liver cancer cases and limited or no information on potential co-exposures. Rice and Boffetta (2001) also remarked that the chloroprene occupational exposure assessment was poor for all published studies and that the statistical power of the available studies was low due to the small number of observed outcomes. Notably, the co-author identifying these limitations, Dr. Paolo Boffetta, was also a contributing author on papers describing the results of the cohort studies in Russia and

Armenia. In the eight years since the Rice and Boffetta (2001) review was published, the limitations of the studies of Chinese, Russian, and Armenian cohorts remain. It is important to recognize that of all the available epidemiological studies, only the original studies of the U.S. cohort from Louisville, Kentucky (Pell 1978, Leet and Selevan 1982) have been updated to address previously identified limitations (Marsh *et al*. 2007a,b).

1.A.1. The Marsh et al. (2007a, b) Study

The Marsh *et al*. (2007a, b) study included all workers (n=12,430) with potential chloroprene exposure at each of four chloroprene production sites from plant start-up date through the end of 2000 (1999 for one site). The sites include two DuPont/Dow Elastomers LLC (DDE) plants in the U.S. (Louisville, KY and Pontchartrain, LA), one DDE plant in Maydown, Northern Ireland (NI), and one Enichem Elastomers France plant in Grenoble, France (FR) (called by the study authors Plants L, P, M and G)[1]. These studies of workers from four chloroprene production sites in the U.S. and Europe provide the largest, most comprehensive and most rigorous investigation of the long-term health effects of exposure to chloroprene conducted to date. The Marsh *et al*. study was designed to overcome most of the shortcomings and uncertainties noted by Rice and Boffetta (2001) and Acquavella and Leonard (2001) that have limited the interpretation of findings from the previously available cohort studies.

The large size of the Marsh *et al*. (2007a, b) cohorts compared with all other epidemiological studies reviewed in the Draft Review is evident in **Table 2**. As shown in **Table 2**, the Marsh *et al*. study comprises 57% of the subjects, 74% of the total person-years at risk, 88% of the lung cancer deaths and 49% of the liver cancer deaths found in the epidemiological studies of chloroprene-exposed populations included in the Draft Review. The Marsh *et al*. study provides a substantial portion of the available epidemiological data on cancer risks associated with chloroprene exposure.

In particular, the Louisville, Kentucky cohort, most recently reported on by Marsh *et al*. (2007a, b), is the largest and most extensively studied chloroprene-exposed worker population evaluated for cancer mortality. The initial analysis of mortality data from this cohort spanned from 1957 through 1974 (Pell 1978). Subsequently, these data were reanalyzed using a different reference population for standardized mortality ratio (SMR) estimation with the addition of a dichotomous cumulative exposure categorization (Leet and Selevan 1982). Marsh and colleagues (2007a,b) advanced the follow-up start date for the cohort from 1957 to 1949 and

[1] Chloroprene production dates for each plant were: L (1942–1972), P (1969–2000), M (1960–1998) and G (1966–2000). In two Plants (L and M), chloroprene production included an acetylene-based process that produced vinyl chloride as a by-product. The Marsh *et al*. studies were completed in 2005 and resulted in the publication of a series of articles on the epidemiology and exposure assessment components (Marsh *et al*., 2007a, b; Esmen *et al*., 2007a-c; Hall *et al*., 2007; Leonard *et al*., 2007).

extended the mortality ascertainment from 1974 through 2000 and incorporated an extensively documented exposure assessment (Esmen *et al*. 2007a, b; Hall *et al*. 2007).

Despite the large size and methodological strengths of the Marsh *et al*. (2007a, b) study, diminished weight is given to this study in the Draft Review based on comments regarding methodological issues or conclusions regarding lung and liver cancer risks among chloroprene-exposed workers. Specific examples of the under-representation of the Marsh *et al*. (2007a, b) study in the Draft Review include:

- Section 4.1.1.3, Summary and Discussion of Relevant Methodological Issues (page 4-15, lines 14-16) states: " In general, the constructed data bases do not include detailed information on the workers' individual habits (e.g., tobacco use, alcohol consumption) and *usually only have limited exposure information* [emphasis added]. These limitations often limit the ability to control for bias due to confounding variables and to assess the potential for misclassification of exposure."
- Section 4.1.1.3 (lines 31-35) states, "Finally, the lack of quantitative exposure assessment is *clearly a limiting factor of most occupational studies* [emphasis added], however, they are still able to contribute to the overall qualitative weight of evidence considerations."
- The Liver Cancer Summary (page 4-18, lines 22-23) states, "Further limitations in these cohorts include the *lack of precise quantitative exposure information"* [emphasis added],
- The Summary of Animal and Tumor Data and Weight of Evidence (Table 4-38) states in the conclusions box, "Methodological limitations of the occupational epidemiological studies (e.g., no available data for some potential confounders which precluded adjustment, limited statistical power due to small sample sizes, and *lack of precise quantitative exposure ascertainment) make it difficult to draw firm conclusions regarding the human cancer data*" [emphasis added].

These statements could erroneously lead the reader to conclude that Marsh *et al*. (2007a. b) inadequately characterized chloroprene exposures. However, in his review of the Marsh *et al* (2007a, b) study, Bukowski (2009) concluded (page 1210):

> *"The best exposure assessment is provided for the four cohorts evaluated by Marsh et al. (2007b). This assessment, which is detailed in several publications,(Marsh et al. 2007b; Esmen et al. 2007a, b, c) used mathematical exposure models based on physical properties, process chemistry, chemical engineering, dispersion physics, historical records on equipment and operating procedures, and personal interviews. Modeling results allowed jobs to be classified into one of eight exposure categories based on roughly order-of-magnitude differences. Summary metrics allowed workers to be classified with respect to cumulative exposure, average intensity of exposure, and duration of exposure. These modeling results were validated against available IH measurements, and this showed a good relationship between measured and predicted exposures (Esmen et al. 2007a). Statistical assessment suggested that there was less than 10% misclassification using this classification scheme (Esmen et al. 2007b).*

The weakest link in the above process appears to be the determination of task/job duration and frequency, which relied on interviews, documented operating procedures, simple experiments, and educated conjecture (Esmen et al. 2007a). However, this limitation is shared by all the reviewed studies. Furthermore, Esmen et al. showed that duration was fairly accurately recalled for major tasks that were performed repetitively throughout long periods of operation (Esmen et al. 2007a)."

The above cited example statements suggest that the comprehensive and rigorous quantitative and qualitative exposure assessment conducted for the Marsh *et al*. (2007a, b) study has not been fairly represented in the Draft Review.

USEPA statements also indicate that the Marsh *et al*. (2007a, b) study was given little weight in arriving at overall conclusions regarding lung and liver cancer risks associated with chloroprene exposure. Most notably, Marsh *et al*. (2007a, b) concluded that workers exposed to chloroprene at the levels encountered in the four study sites did not have elevated risks of mortality from any of the causes of death examined, including all cancers combined and cancers of the lung and liver. These are the sites of *a priori* research interest, a conclusion not clearly expressed in the Draft Review.

1.A.2 Other Epidemiological Studies Considered

In the Draft Review, each published epidemiological study is considered as independent and does not acknowledge that cohort studies that have been updated should be discarded in favor of the most recently reported analytic results[2]. The results reported in earlier reports, particularly those referenced from the Pell (1978) and Leet and Selevan (1982) studies are no longer applicable to describe the mortality experience of the Louisville cohort. This cohort is most comprehensively described by the 50-year follow-up conducted for the Marsh *et al*. (2007a, b) study. Therefore, analytic results reported by Leet and Selevan (1982) should be removed from consideration (Tables 4-1, 4-10, and 4-11) when drawing conclusions regarding the carcinogenic potential of chloroprene. Additionally, reference to the Leet and Selevan (1982) study to support interpretations of a positive association between chloroprene exposure and both liver and lung cancer mortality, most specifically Sections 4.7.1 and 4.7.2.1.1 and Table 4-38, should also be removed from the Draft Review.

The justification for positive associations between chloroprene exposure and liver and lung cancer cited in the Draft Review relies extensively on the overall and stratified results of three cohort studies involving workers in China (Li *et al*. 1989), Russia (Bulbulyan *et al*. 1998), and Armenia (Bulbulyan *et al*. 1999), if the Leet and Selevan (1982) study is removed from consideration in the weight-of-evidence assessment. The major findings of the Russian and

[2] Cohort studies comprise a set of data points that provide a distribution of results to address a hypothesized exposure-disease association (Checkoway *et al*. 2004, p. 332-333). This consideration assumes that each study cohort is an independent sample and that analytic results are not correlated between studies. In practical terms, established guidelines for qualitative reviews prescribe that there should be no overlap of included studies (Greenland and O'Rourke, 2008). *In effect, only the most recent results from any cohort study should be considered in the Toxicological Review*.[emphasis added] Applying this standard approach, the resulting literature should comprise 7 published studies conducted in 9 cohorts.

Armenian cohorts were reprinted by Zaridze and colleagues (2001). The limitations of these three studies are extensive and have been noted in the peer-reviewed literature by Rice and Boffetta (2001), Acquavella and Leonard (2001), and Bukowski (2009). None of these limitations has been corrected or addressed by the investigators in subsequent analyses of these cohorts since their original publication.

The study of Chinese workers (Li *et al*. 1989) suffers from insufficient statistical power, inadequate reference population mortality rates for statistical comparisons, and a lack of adjustment for important factors associated with increased cancer risk especially liver cancer risk in China. The researchers ascertain cohort mortality for a 14-year period from 1969 through 1983 and report six deaths due to liver cancer and two deaths due to lung cancer among 1,213 workers. However, they only used local area mortality rates as a reference population to estimate expected numbers of cancer outcomes during a three year period (1973 to 1975). This method for estimating expected mortality from a brief interval of the total cohort follow-up period may underestimate the expected numbers of liver and lung cancer deaths for the cohort. Li and colleagues report 2.5 and 0.4 expected liver and lung cancer deaths, respectively, among all cohort members followed between 1969 and 1983. The limited number of observed liver and lung cancer deaths divided by the very small expected numbers produced excess but unstable standardized mortality ratios (SMR).

Estimates for liver and lung cancer incidence are high among Chinese men (in 2002, liver cancer mortality was 38 cases per 100,000 persons per year, and lung cancer mortality was 42 cases per 100,000 persons per year) and women (liver cancer, 14 cases per 100,000 persons per year, and lung cancer, 19 cases per 100,000 persons per year) (Parkin *et al*. 2005). In the most high-risk areas of China, one in ten people die of liver cancer (Hsing *et al*. 1991). The major causes of liver cancer in China are chronic infection with hepatitis B virus (HBV) and aflatoxin B_1, in addition to the rising prevalence of alcohol consumption and tobacco smoking (Chen *et al*. 2003, Stuver and Trichopoulos 2008, Lee *et al*. 2009). Therefore, an observational mortality study for liver cancer within this population should attempt to control for these confounding risk factors. Moreover, these risk factors enhance the need to determine histopathology of liver cancers in this study population, as recommended by Rice and Boffetta (2001), to determine whether liver cancers are consistent with those most often associated with occupational exposures (Lloyd 1975).

Similar to the Li *et al*. (1989) study, the reference population used for the Russian cohort study likely underestimates the liver cancer mortality for the Moscow population for the observed cohort follow-up period from 1979 to 1993. Bulbulyan and colleagues (1998) calculated expected numbers of liver cancers using mortality and incidence rates for the general population of Moscow during the years 1992 to 1993. It is important to note that cancer mortality data from 36 European countries, including the Russian Federation, show that liver cancer mortality rates among women increase from 1960, peak during the late 1970s, and demonstrate a general decline to their lowest levels during the early 1990s, the period chosen for the study's reference mortality rates (Levi *et al*. 2004).

For the Armenian cohort study, expected cancer incidence rates were obtained from the Armenian Cancer Registry for the period 1979 through 1990. Bulbulyan and colleagues note

that the Armenian cancer registry is incomplete and may be misclassified in regard to the histopathology of reported liver cancers for the general population. These characteristics of the selected reference population may result in an underestimate of the expected liver cancer incidence and mortality used to calculate standardized ratio estimates. Moreover, the accuracy of reported statistics is questionable for the Armenian cohort (Bulbulyan *et al*. 1999, Zaridze *et al*. 2001). **Table 3** presents the exact Poisson confidence intervals based on the reported SMR or SIR and the number of observed cases provided by Bulbulyan and colleagues. We calculated the expected number for the standardized ratio by dividing the observed cases by the SMR or SIR. The 95% confidence limits were estimated using the method from Breslow and Day (1987) that was also referenced by Bulbulyan *et al*. (1999). The recalculated 95% confidence limits are attenuated from those reported in the original publications. This systematic error raises questions regarding the validity of the statistical approach used by Bulbulyan and colleagues for the analyses. In light of the small numbers and the likelihood that chance may be an explanation for these estimates, the inaccurate and imprecise numbers reported in the Bulbulyan *et al*. (1999) and repeated in Zaridze *et al*. (2001) should be viewed skeptically.

The Russian and Armenian cohorts also suffer from inadequate consideration of other major risk factors that contribute to liver cancer. In the populations represented in these cohorts, there is a high incidence of alcoholic cirrhosis, a well known precursor for liver cancer (London and McGlynn 2006). Eleven deaths from cirrhosis of the liver (3 in males and 8 in females) were recorded for the Russian cohort. In the Armenian cohort, 32 cases of cirrhosis of the liver were reported (27 in males and 5 in females). Alcohol consumption and smoking are well known risks factors for liver cancer, and these factors were not adjusted for in the eastern European cohort studies (Keller 1977, Makimoto and Higuchi 1999, Lee *et al*. 2009). A recent report by the World Health Organization (WHO 2009) found a prevalence of 70% and 27% for current tobacco use among Russian men and women, respectively, and noted high levels of alcohol consumption for the general population. The prevalence of current tobacco use among Armenian men is also very high at 55% (WHO 2009). These highly prevalent and well-known risk factors for liver and lung cancers should be thoroughly considered when interpreting the small number of observed and expected cases reported in the occupational cohort studies.

1.A 3 Summary

Marsh *et al*. (2007a, b) is the largest study of potential cancer outcomes with the most complete documentation of exposure that has been published. If a true causal association existed with chloroprene, the likelihood that this study would have failed to detect an effect is low, given its multiple cohorts and dozens of analyses. The Marsh *et al*. (2007a, b) study was designed and conducted to address many of the limitations inherent in the other epidemiological studies for chloroprene. As detailed in a peer-reviewed assessment (Bukowski 2009), the Marsh *et al*. (2007a, b) study clearly has the most strength and should receive the highest relative weight when conclusions are drawn regarding potential effects following chloroprene exposure. Due attention should be given to the published interpretation by Marsh and colleagues that exposure to chloroprene does not elevate liver and lung cancer mortality risks. This conclusion should be the prevailing inference derived from epidemiologic research of chloroprene.

Outside of the Marsh *et al*. (2007a, b) study, the evidence of carcinogenicity of chloroprene is based on four studies: Li *et al*. (1989), Bulbulyan *et al*. (1998, 1999), and Colonna and Laydevant (2001). Li *et al*. (1989) was the only study to note an association between chloroprene exposure and lung cancer, based on only two cases. The two Bulbulyan *et al*. (1998, 1999) studies and the Li *et al*. (1989) study reported associations with liver cancer and chloroprene based on ten or fewer cases in each cohort. These studies have extensive methodological limitations that were not adequately considered (and in accordance with USEPA criteria for epidemiological data quality) in the Draft Review. The Draft Review does not take these limitations into account and thus inappropriately uses the results from these previous studies to infer a positive correlation between chloroprene exposure and liver and lung cancers.

The large size of the Marsh *et al*. (2007a, b) study compared with the other epidemiological studies reviewed in the Draft Review (**Table 2**) demonstrates that the results of this study should receive substantial weight (i.e., strength of evidence) in drawing conclusions regarding cancer risk associated with chloroprene. As noted in Attachment A (**Table A1**), only the Marsh *et al*. (2007a, b) cohorts had quantitatively estimated historical exposures to chloroprene and was the only study to conduct detailed exposure-response modeling. Clearly, the Marsh *et al*. (2007a, b) study provides a substantial portion of the available epidemiological evidence for potential cancer risks associated with chloroprene exposure. The conclusions of this study should, in a weight-of-evidence analysis that considers the limitations of previous epidemiological investigations, prevail, i.e., the overall weight-of-evidence does not support the conclusion that chloroprene is "likely to be carcinogenic to humans".

1.B. Interpretations of standardized ratios failed to consider the impact of low expected counts in the Draft Review from three cohort studies (China, Russia, and Armenia).

Despite the earlier documented general rates for liver and lung cancers in the Chinese, Russian, and Armenian populations, it is surprising that reported expected liver and lung cancer mortality and incidence counts are very low for the cohort studies in these countries. Many of the expected counts are below 1.0 and most are below 2.0. This indicates either inaccurately reported population rates or a questionable approach to estimating expected deaths using the selected population mortality rates. Additionally, these studies may have insufficient follow-up periods for the cohorts that could also contribute to low expected counts. Previous reviews have critiqued these three studies, especially the Chinese study, for inadequate descriptions of the source population rates used to calculate SMRs and SIRs (Rice and Boffetta 2001).

There is also an important methodological concern for the interpretation of excess risk based on SMR and SIR estimates when considering standardized ratios based on expected values less than 2. As Checkoway and colleagues (2004) advise,

> "In most instances, the SMR [and SIR] for an entire cohort for a particular disease should be regarded with caution when the expected value (not the observed) is less than 2 because such a result may be misleading, except when the disease is very rare in non-exposed populations (e.g., malignant mesothelioma)." (p. 146)

Random chance may influence the occurrence of a limited number of observed outcomes that may overwhelm the very small estimate of expected health outcomes in a ratio measure. In practical terms, observed cancer deaths can only be whole number counts. There is little plausibility for an estimate of 0.4 expected cancers in a cohort of workers followed for a limited time, and there are many standardized ratios reported in the three cohort studies (China, Russia, and Armenia) for which the expected number is less than 1.0. As shown in **Table 4** and **Table 5**, nearly every standardized ratio measure representing an increased risk as interpreted in the Draft Review is generated when the expected denominator is less than 2.0. This indicates that imprecise and unstable estimates of the SMRs and SIRs as reflected by wide confidence intervals reported for results from the China, Russia, and Armenia cohort studies may be biased by either systematic or random errors. At a minimum, a cautious approach should be considered when interpreting the excess risk estimates associated with these very low expected counts.

1.C. Assessing causality failed to apply methods recommended by the *Guidelines for Carcinogen Risk Assessment* (USEPA 2005) in the Draft Toxicology Review.

The weight of evidence approach in the current Draft Review fails to consistently apply methods recommended by the *Guidelines for Carcinogen Risk Assessment* (USEPA 2005) in regard to causal inference. Specifically, the *Guidelines* state,

> "In assessing the human data within the overall weight of evidence, determination about the strength of the epidemiological evidence should clearly identify the degree to which the observed associations may be explained by other factors, including bias or confounding." (p. 2-4).

This recommendation explicitly states that available epidemiologic quantitative results be evaluated for potential bias due to systematic errors (i.e. bias, misclassification, and confounding) and random errors (i.e. the role of chance). The impacts of these potential sources of bias for excess quantitative risks reported in the Chinese, Russian, and Armenian cohorts are not reflected in the current Draft Review. As noted, there is a consistent conclusion in previous reviews of published chloroprene epidemiological studies (Rice and Boffetta 2001, Acquavella and Leonard 2001, Bukowski 2009) that the studies indicating a positive association are of insufficient quality to infer a causal relationship between chloroprene exposure and cancer outcomes of interest. This caution is further augmented by the lack of a detailed quantitative exposure assessment for chloroprene in many of the cohorts for which positive associations are reported.

Further, the Draft Review states that a number of methodological limitations exist that "may preclude drawing firm conclusions regarding" the criteria for causality (page 4-70). Causal criteria are most often adapted from a list of guidelines commonly referred to as "Hill's criteria" for judging causality (Hill 1965). Although these guidelines cannot be used as a complete quantitative checklist, it is also incorrect to select a subset of the criteria without justification of the characteristics of evidence necessary to satisfy each criterion (Weed 2005). Hill noted in his 1965 speech presenting his causal guidelines that,

> "Nevertheless, whether chance is the explanation or whether a true hazard has been revealed may sometimes be answered only by a repetition of the circumstances and the observations."

In the current Draft Review, important considerations for the role of random chance are missing in the face of a limited number of findings based on limited studies. Additionally, a thorough evaluation of the impact that systematic biases related to the selection and reporting of imprecise excess risk estimates is not presented.

1.D. USEPA interpretations of the potential for lung and liver cancer risks of chloroprene based on the Marsh *et al.* (2007a, b) study did not fully consider the impact of inordinately low death rates for lung and liver cancer among workers in the baseline categories.

In the Draft Review, the Marsh *et al*. (2007a, b) study data is interpreted as supporting the elevated lung and liver cancer risks purported in previous cohort studies, including purported evidence of an exposure-response relationship for chloroprene exposure and liver cancer. However, the original investigators of the Marsh *et al*. (2007a, b) study concluded that exposure to chloroprene at the levels encountered in the four study plants did not elevate lung or liver cancer risks, in contrast to the previous epidemiological studies (Marsh *et al*., 2007a,b). These major differences in interpretation stem from the oversimplification presented in the Draft Review of the methodological issues associated with low baseline deaths rates used for internal mortality comparisons.

In the Marsh *et al*. (2007a, b) study, low baseline death rates were observed and were attributed to a healthy worker effect bias in the Draft Review. However, the Draft Review did not consider the many other alternative explanations discussed in detail by the investigators of the Marsh *et al*. (2007a, b) study. In fact, investigators of the Marsh *et al*. (2007a, b) study concluded that chance or the healthy worker effect were unlikely explanations for the spuriously low baseline rates (Marsh *et al*, 2007b) for reasons discussed below. Bukowski (2009) also concluded that several factors argue against a strong healthy worker effect, especially in the predominant Louisville cohort examined in the Marsh *et al*. (2007a, b) study. The lack of consideration of anomalous baseline rates in the Draft Review leads to the incorrect conclusion that the risk of death from lung and liver cancer was elevated in the Marsh *et al*. (2007a, b) cohort and that there was evidence of an exposure-response relationship for chloroprene exposure and liver cancer.

1.D.1. Study Findings for Lung Cancer

In the Draft Review, it is concluded that the available epidemiological studies provide some evidence of an exposure-response relationship for chloroprene exposure and lung cancer and that the Marsh *et al*. (2007a, b) study observed little evidence of elevated respiratory system (lung) cancer rates in the Louisville cohort and no evidence of an exposure-response relationship. As shown in **Figure 1**, no elevations were observed using both external (SMR) and internal (relative risk [RR]) comparisons. The Louisville plant was the largest Marsh *et al*. (2007a, b) study cohort examined, yielding large numbers of observed lung cancer deaths and correspondingly precise SMRs and RRs. **Figure 1** also shows that SMRs for all non-baseline cumulative

chloroprene exposure categories were less than 1.00, indicating no evidence of excess lung cancer risk. The only excess risk appeared for the second highest exposure category (RR=1.32) and this was not statistically significant. Given the SMRs of less than 1, this single RR excess represents a misleading artifact that was caused by the statistically significant lower death rate for lung cancer in the unexposed, baseline category (SMR=.71, $p<.05$), that is, for each non-baseline category, RRs are roughly the ratio of the SMR in the non-baseline to baseline categories.

In the Marsh *et al*. (2007a, b) study of the Grenoble, France cohort (**Figure 2**), a similarly low baseline death rate for lung cancer coupled with small excess rates in the non-baseline categories, produces the misleading appearance of more pronounced elevations in RRs across the non-baseline categories. Note that none of the RRs was statistically significant and there is little evidence of a monotonic exposure-response relationship. For the small cohort, the number of observed lung cancer deaths was very small producing unstable SMRs and RRs.

The most misleading impressions presented in the Draft Review about excess risks and exposure-response relationships for lung cancer occurred for the Marsh *et al*. (2007a, b) study of the Maydown, Ireland and Pontchartrain, Louisiana cohorts (**Figure 3** and **Figure 4**). The lung cancer death rates for the baseline categories were spuriously low, SMRs=0.54 and 0.40, respectively ($p<0.05$). Therefore, SMRs this low and, for Maydown, statistically significantly low, cannot be due simply to bias from the healthy worker effect as concluded in the Draft Review. The rationale for this statement is further discussed below.

Lung cancer SMRs were below 1.0 for the Maydown and Pontchartrain cohorts, with the exception of a slight excess in the highest exposure category for Maydown (SMR=1.13), indicating no evidence of elevated lung cancer rates. The perception of elevated RRs for lung cancer in these two cohorts is due simply to the unusually low death rates in the baseline category.

1.D.2 Study Findings for Liver Cancer

Throughout the Draft Review, evidence of liver cancer in relation to chloroprene exposure at the Louisville plant is cited as evidence of an exposure-response relationship, which appears to drive the overall conclusion that chloroprene is carcinogenic in humans. Based on the data presented in **Figure 5** in the "Liver Cancer Summary" (page 4-18, lines 2-5), the Draft Review states that, "Although no statistically significant increase in risk of liver cancer was detected in the Louisville plant (Marsh *et al*., 2007b), the relative risk increased with increasing cumulative exposures indicating a dose-response trend." There are several major issues with USEPA's interpretation of the data in this figure.

First, there is absolutely no evidence of a statistically significant, monotonic increase in SMRs or RRs in these data, despite the problems of the low baseline rate. Further, none of the non-baseline category-specific SMRs or RRs was statistically significant. Second, the appearance of a 2 to 5-fold increase in liver cancer risk indicated by the RRs is due simply to the spuriously low baseline death rate for liver cancer (indicated by the 57% deficit in liver cancer deaths [SMR-0.43]). As noted above for lung cancer, SMRs this low cannot be due simply to bias from the health worker effect, as concluded in the Draft Review. Third, the SMRs and RRs for liver

cancer at Louisville in relation to cumulative chloroprene exposure are based on small numbers of deaths, especially the two deaths associated with the baseline category. This produced relatively imprecise SMRs and RRs as indicated by the very wide confidence intervals.

1.D.3 Alternative Explanations for the Low Baseline Rates in the Marsh et al. (2007a, b) Study

As noted above, USEPA's attribution of all low baseline death rates observed in the Marsh *et al*. (2007a, b) study to healthy worker effect bias does not consider the many other alternative explanations discussed in detail by the investigators of the Marsh *et al*. (2007a, b) study. This section provides a discussion of alternative explanations for the spuriously low baseline rates.

The main strength of the Marsh *et al*. (2007a, b) exposure-response analyses was the use of national and local county mortality comparisons and robust statistical modeling of internal cohort rates. The strengths of the internal study group comparison are that it will usually reduce the healthy worker effect and that it allows direct comparison of RR across strata. However, internal comparisons can be unstable when the study population is small and/or the disease under study is rare (producing wider confidence limits). Internal comparisons may also be misleading if workers included in the baseline category (i.e., least exposed) have different underlying cancer risks than workers in the exposed groups. On the other hand, external comparisons based on regional rates have the strengths of being able to adjust for geographic variability in social, cultural and economic factors related to disease (Doll, 1985) and are generally very stable. The disadvantages of the external comparison group are inability to adjust for the healthy worker effect and difficulty in comparing standardized mortality ratios between groups when their confounder distributions differ (Checkoway *et al*., 2004).

When Marsh *et al*. (2007a, b) compared death rates of study subjects to those of the general population residing in the surrounding counties of each study plant, they observed many deficits in deaths from all cancer combined, lung and liver cancer. While the largest deficits often occurred among unexposed workers, deficits in deaths remain among workers in the non-baseline exposure categories. This pattern of findings by exposure category in the external population-based SMRs led to elevated RRs of disease when rates for non-baseline categories were compared to the baseline (unexposed) rates. For example, as shown in **Figure 4**, for lung cancer by cumulative exposure to chloroprene in Plant P, an RR of 2.32 (95%CI=.30-21.83) for the highest exposure category (16.20+ part per million-years), or an apparent 2.32-fold excess, results because a small 15% deficit in deaths in the highest exposure category (SMR=.85, 95%CI=.23-2.18) is essentially being compared to a exceedingly large 60% deficit in the baseline category (SMR=0.40, 95%CI= 0.08-1.18).

Thus, the question arises *as to whether the ratio of small to large deficits in deaths (essentially, but not exactly, what is expressed via RRs) should be interpreted as a meaningful "excess" in deaths?* This enigmatic feature of exposure-response analyses created by inordinately low baseline rates has been observed in other major occupational cohort studies, such as the cohort studies of formaldehyde (Blair *et al*., 1986; Hauptmann *et al*., 2003; 2004) and acrylonitrile (Blair *et al*., 1998) workers conducted by the National Cancer Institute (NCI), and has stimulated reanalysis and reinterpretation of the NCI cohort data (Marsh *et al*., 2001; Marsh and Youk,

2004, 2005). Although RRs for the cancer sites and exposure measures considered were elevated in many non-baseline categories due to the low baseline rates, Marsh *et al*. (2007a, b) observed no consistent evidence that RRs were positively associated with increasing exposure in any of the study plants.

There are at least two possible explanations for the large differences in the cancer relative risks in chloroprene cohorts when internal or external comparison rates are used. The first is that internal comparisons produce more valid results because selection bias stemming from the "healthy worker effect" can reduce the putative effect of high exposure to chloroprene (or vinyl chloride) when external comparison rates are used. The healthy worker effect is evident in this population by the low RRs for all causes of death for chloroprene-exposed (SMR=0.71, 95% CI=0.69-0.73) and chloroprene-unexposed workers (SMR=0.88, 95% CI=0.69-1.10). However, the selection for workers who are healthy at time of hire is usually more relevant for cardiovascular and nonmalignant respiratory diseases than lung cancer, which has a relatively sudden onset, short survival time and high case-fatality rate (Enterline, 1976).

A second explanation is that the external comparisons produce more valid results because the unexposed group has a different underlying cancer risk than the exposed group. As shown above, the risk in the highest exposure category when internal comparisons are used may simply be the result of an unusually low lung cancer death rate among workers in the unexposed baseline category. In fact, had the death rates for all cancer, respiratory system or liver cancer among the unexposed workers been closer to or equal to those of the general regional populations from which the four plant workforces were drawn, the internal RRs calculated for quartiles of chloroprene exposure across the total cohort would probably have been uniformly near or less than 1.0.

The very low SMRs for all cancer, lung and liver cancer, especially among unexposed workers, are puzzling given that Marsh *et al*. (2007a, b) used regional standard population rates. Although a small percentage of deaths (estimated at about 5%) may have been missed among transferees in Plant P and among subjects who emigrated in Plants M and G (Marsh *et al*., 2007a), under-ascertainment of deaths is an unlikely explanation for these low SMRs. Also, because regional rates can help adjust for the social, cultural and economic factors related to diseases such as lung cancer, and even help to adjust for geographic variability in tobacco use (Doll, 1985), it is difficult to postulate what non-occupational factors may have had such a profound influence on the cancer mortality experience of this cohort.

The Marsh *et al*. (2007a, b) investigators hoped that an additional model adjustment for worker pay type (a correlate of education/socioeconomic status) and thus, smoking history, might help to explain the inordinately low and often statistically significant baseline SMRs for all cancers combined and respiratory system cancers found for each study plant in the baseline categories of each exposure measure. For example, if subjects at risk in the baseline exposure categories were lighter smokers than subjects at risk in the non-baseline categories, this would negatively confound baseline SMRs for respiratory system cancer relative to non-baseline SMRs and positively confound the corresponding non-baseline RRs. To a lesser extent, the same pattern could occur for all cancers combined. However, with the possible exception of Plant G, where pay type-adjusted RRs for all cancers combined were uniformly less, suggesting positive

confounding by smoking, the additional adjustment for worker pay type did not materially alter the pattern of RRs for all cancer and respiratory system cancer found in the unadjusted models.

With the possible exception of liver cancer in Plant L (based on small numbers of death), chance alone does not appear to be an explanation for the cancer deficits observed among unexposed workers in this study. U.S. and regional rate-based SMRs (and RRs) for all cancers and respiratory system cancer in all categories of the chloroprene exposures examined were based on sufficiently large numbers of observed deaths to provide stable risk estimates, and deficits were generally consistent across the chloroprene exposure categories considered. Also, the general quality of the follow-up and cause of death ascertainment in this study ruled out under-ascertainment of cancer deaths as a reason for the deficits. Given the absence of a viable explanation derived from the available study data, what remains is the possibility that some heretofore unknown selection factors for low cancer incidence or mortality were operating on the unexposed subjects in this cohort, or that some type of protective effect for lung cancer arose from a particular exposure or combination of exposures encountered at the study plants. Without further formal investigation of this phenomenon in the chloroprene cohort, the reason(s) for the marked deficits in cancer in unexposed workers will remain unknown.

Bukowski (2009) also discusses the inordinately low baseline rates for lung and liver cancer observed in the Marsh *et al*. (2007a, b) study and the extent to which the healthy worker effect may have affected these findings. For liver cancer in relation to chloroprene exposure, Bukowski (2009) states, "the internal RRs for liver cancer then to increase with higher levels of cumulative exposure (RR 3.3–5.1), but these increases were neither significant nor monotonic." As with the French and Irish cohorts, the mortality rates for the baseline exposure groups used in the RR analysis were much lower than the rates for the local area (liver cancer SMR 0.43–0.61), making the internal references weak baselines for comparison."

As for the role of the healthy worker effect on the low baseline rates, Bukowski (2009) states,

> *"The HWE seems to have impacted the four cohorts evaluated by Marsh et al., but several factors argue against a strong distortion of cancer SMRs within the results reported for the Louisville cohort. First, the associations for all deaths or deaths from cardiovascular disease are only moderately depressed (SMR 0.7–0.8), suggesting only a moderate HWE. (Marsh et al. 2007a) This is consistent with expert opinion regarding the typically modest nature of the HWE. (Enterline et al. 1988; Nicholson 1988) Second, the advanced age of this cohort, which dated back to when operations began in 1942 (with follow-up to 2000), would tend to decrease or even reverse the HWE. (Axelson 1988; Doll 1988, McMichael 1988; Monson 1988; Gun et al. 2004; Archer 1995)"*

In summary, the analysis of the cancer mortality experience of the chloroprene cohorts (Marsh *et al*. 2007a, b) provides no evidence that exposure to chloroprene at the levels encountered in the four study plants increases the risk of death from all cancers or the sites of *a priori* interest, lung and liver. The findings of Marsh *et al*. (2007a, b) based on external comparisons using regional rates produced exposure category-specific risks very different than those based on internal rates due largely to inordinately low death rates among workers in the unexposed categories. The

original Marsh *et al*. investigators concluded that chance or selection bias in the form of the healthy worker effect were unlikely explanations for these differences.

1.E. Additional analyses provided by the Marsh *et al.* (2007a, b) investigators further support the lack of an association between lung and liver cancer risks and chloroprene exposure.

Additional analyses are provided here that have been conducted by the original investigators and were mentioned but not documented in the Marsh *et al*. (2007a, b) study publications. One analysis is the metric time since first exposure to chloroprene. The SMRs provided are computed for exposed workers only. As seen in **Table 6**, there is no evidence of an association between liver cancer and chloroprene exposure. There are no increased risks of liver cancer for increasing categories of time first exposure. The SMR for workers who were followed for 40 or more years since first exposure was essentially unity.

Lagged measures of cumulative exposure to chloroprene show no evidence of an exposure-response relationship for liver cancer and increasing levels of chloroprene exposure, as shown in **Figure 6**, which is similar to the unlagged analysis. The 15-year lag led to SMRs that were very similar in the three highest levels of cumulative exposure, essentially flattening any exposure-response curve.

In summary, the researchers of the Marsh *et al*. (2007a, b) study maintain that the available data for liver cancer in relation to chloroprene exposure from the Marsh *et al*. (2007a, b) study Louisville cohort provide no evidence of an exposure-response for chloroprene and liver cancer.

1.F. Vinyl chloride exposure as a possible confounder of the association with chloroprene exposure and liver cancer in the Marsh *et al.* (2007a,b) study is not supported given the lack of correlation between chloroprene and vinyl chloride exposure.

On page 4-14, of the Draft Review, USEPA states:

> "*The authors also conducted internal analyses of cancer mortality and vinyl chloride exposure (the primary co-exposure in this study) at the Louisville plant. They found an inverse association (many of them statistically significant) between risk of both respiratory and liver cancer in relation to vinyl chloride exposures. In fact, the vast majority of respiratory and liver cancers occurred among workers who were unexposed to vinyl chloride. If vinyl chloride is a negative confounder of the association between chloroprene and liver cancer, then the reported association between chloroprene and liver cancer would be an underestimate of the association adjusted for vinyl chloride. Given this, it is highly unlikely that confounding by vinyl chloride could explain the associations observed between chloroprene and these cancers. In addition, the authors reported that there was no correlation between cumulative exposures to vinyl chloride and chloroprene among these workers."*

USEPA statements suggesting that vinyl chloride exposure may be a negative confounder of the chloroprene and liver cancer association are not justified for two main reasons:

(1) The inverse associations between vinyl chloride exposure and liver cancer are based on only two deaths spread across three non-baseline exposure categories, providing limited data to support an association. This evidence is very imprecise since 15 of the 17 liver cancer deaths in Louisville were unexposed to vinyl chloride. In fact, many of the RRs estimated for these categories containing only one or zero liver cancer deaths were derived as non-robust median unbiased estimates using exact conditional logistic regression.

For example, Marsh *et al*. (2007b) shows the sparseness of the data and instability of the RRs for the non-baseline categories in the exposure-response analysis for vinyl chloride exposure and liver cancer using both average intensity of exposure and cumulative exposure to vinyl chloride (**Table 7**).

(2) Because Marsh *et al*. (2007b) clearly show in their publication that chloroprene and vinyl chloride exposures are not correlated, the issue of potential confounding of the chloroprene and liver cancer association is rendered moot. This lack of association between chloroprene and vinyl chloride exposure in Louisville and Maydown can be seen from the scattergrams taken from Marsh *et al*. (2007b) (**Figure 7**).

While confounding by vinyl chloride exposure was not an issue in the Marsh *et al*. (2007a, b) study, the investigators were concerned about the possibility that vinyl chloride might modify the effect of chloroprene in regards to liver cancer risk. As mentioned in the Marsh *et al*. (2007b) publication,

> *"While not shown, our analysis of mortality among Plant L and M workers in relation to the four composite exposure measures, chloroprene with VC_AIE (average intensity of exposure), chloroprene with VC_Cum (cumulative exposure), chloroprene without VC_AIE and chloroprene without VC_Cum, produced risk estimates similar to those based on the marginal chloroprene exposure measures (i.e., exposure to chloroprene regardless of vinyl chloride exposure) and none of the composite measures revealed evidence of increasing cancer risks with increasing exposure."*

The Marsh *et al*. (2007a, b) investigators have provided in **Table 8** the actual data used in the above analysis. The SMRs were computed using local county rates. These data demonstrate that the Marsh *et al*. (2007a, b) study revealed no evidence that vinyl chloride exposure either acted as a confounder of the chloroprene and liver cancer association or modified the effect of chloroprene exposure.

While vinyl chloride is not a confounder in the Marsh *et al*. (2007a, b) study, Acquavella and Leonard (2001) note the following regarding the Chinese and Armenian cohort studies:

> *"After the Symposium, we learned from investigators working on the industry sponsored chloroprene epidemiology study that the acetylene manufacturing process for*

chloroprene produces vinyl chloride (VC) as a by-product. VC is formed from a side reaction between acetylene and hydrochloric acid in the synthesis of monovinyl acetylene (MVA). VC (at 0.1% yield) is then stripped from the MVA feedstock and vented to the atmosphere. Approximately 0.17 pounds of vinyl chloride are formed for every 100 pounds of chloroprene. Potential exposures to VC will occur at each sampling point, during line changes or cleaning, or during preparation for maintenance for strippers such as unplugging. The solutions (MVA in either acetone or DMF) are kept cool, which should reduce the apparent VC vapor pressure. It is doubtful, however, that this would have significantly reduced potential exposures. From the empirical VC exposure determinations in the purge and sample technique that was used to obtain product quality control samples, estimates of VC exposures in the processes considered may be calculated with varying degrees of sophistication. While exposure reconstruction has not yet been completed in this study, initial estimates suggest that VC exposures in the acetylene process of producing MVA could have been as high as 50 to 750 PPM. This information may be particularly relevant to the interpretation of the Chinese and Armenian chloroprene epidemiology studies since these plants used the acetylene process for the entire period of their respective studies."

This suggests that results observed in these studies may be impacted by the manufacturing process since different starting materials and reaction byproducts are involved. To fully appreciate the hazards of chloroprene, special attention is needed to understand the manufacturing process in use, particularly for the epidemiological studies.

1.G. Statements made on pages 4-13 and 4-14 of the Draft Review concerning the attempt by Marsh *et al*. (2007a, b) to adjust for potential confounding by smoking using the correlated pay type variable are incorrect.

In the Marsh *et al*. (2007a, b) study of the Louisville cohort, the investigators attempted to roughly adjust RRs for lung cancer for potential confounding by smoking using the surrogate variable "worker pay type (blue/white collar)," because it was found in the general mortality analysis (Marsh *et al*., 2007a) that pay type was a risk factor for lung cancer (i.e., higher risk in blue collar workers). Also, pay type is a rough surrogate for education and socioeconomic status, which are highly correlated with smoking status in both the U.S. and Europe (NIOSH 2002).

On Page 4-14, lines 3-5 of the Draft Review state "It is impossible, however, to discern whether this surrogate resulted in control for smoking or resulted in an over-adjustment since work status is so highly correlated with chloroprene exposures". The Marsh *et al*. (2007a, b) investigators respectfully disagree with this statement. For one, if a variable such as pay type is to act as a potential confounding factor for the association with chloroprene exposure and lung cancer, it must be both a risk factor for lung cancer *and* associated with chloroprene exposure. If pay type is not differentially distributed across chloroprene exposure levels then it cannot create different effects at different chloroprene exposure levels, and thus no confounding bias will result. Second, the Marsh *et al*. (2007a, b) investigators are not sure of the basis for the claims that "work status is so highly correlated with chloroprene exposure". No data to support this claim are provided in the Marsh *et al*. (2007a.b) publication. Third, had pay type been so highly

correlated with chloroprene exposure as to cause a near-singularity issue in the estimation of the regression coefficients, the relative risk models would not have converged.

As indicated in Marsh *et al*. (2007b), additional adjustment for pay type had little effect on the lung cancer RRs for either cumulative chloroprene exposure or average intensity of chloroprene exposure. This suggests that pay type was in fact not differentially distributed across chloroprene exposure level categories. While these data were not shown in the Marsh *et al*. (2007b) publication, they are presented in **Table 9** for clarification.

1.H. Summary

Taken together, the referenced epidemiologic studies in the Draft Review describe evidence that should be viewed cautiously. Once reconsidered in light of their previously acknowledged limitations, we do not believe a clear causal inference of liver and lung cancers due to occupational chloroprene exposure can be made. It is facile to state that more definitive studies are needed, particularly those with longer follow-up, and that more complete evaluations are needed of the exposed cohorts in China, Russia, and Armenia. However, no additional analyses from these cohorts have been reported since 2001 making it is difficult to infer that a clear causal association between occupational chloroprene exposure and liver and lung cancer has persisted since the original publications. An implied construct in causal inference is that a "true" finding of excess risk will be present in multiple studies conducted under different conditions at different times, i.e. if the etiologic relationship is causal then it will be replicated especially in larger studies. The scope of epidemiologic evidence presented in the Draft Review relies on several small studies reporting limited numbers of cancer events that may indicate excess risk only on the basis of ratio measures produced by very small expected mortality and incidence counts or inadequately documented exposure-response analyses.

Giving equal weight to a large robust epidemiological study and several low quality, low power studies is an inappropriate and misleading representation of the strength of the most conclusive of the available epidemiological studies. Although the Marsh *et al*. (2007a, b) study has limitations that are typical of all observational epidemiologic studies, it is the largest study of potential cancer outcomes with the most complete documentation of exposure that has been published. If a true causal association existed with chloroprene, it is difficult to imagine a scenario in which this study would have failed to detect it given its multiple cohorts and dozens of analyses. This study was designed and conducted to address the limitations noted in the studies from which the Draft Review derives it inferences for positive associations between chloroprene exposure and liver and lung cancers. As detailed in a peer-reviewed assessment (Bukowski 2009), the Marsh *et al*. (2007a, b) study clearly has the highest relative strength base on the criteria for evaluation listed in the *Guidelines for Carcinogen Risk Assessment (USEPA 2005).* Due attention should be given to the published interpretation by Marsh and colleagues that exposure to chloroprene does not elevate liver and lung cancer mortality risks. This conclusion should be the prevailing inference derived from epidemiological research of chloroprene.

2. Issue for Resolution: USEPA Review and Integration of the Genotoxicity and Mode of Action (MOA) Data for Chloroprene

The hypothesized MOA for chloroprene described in the Draft Review is that it "acts via a mutagenic mode of action involving epoxide metabolites formed at target sites" and further states that "this hypothesized mode of action is presumed to apply to all tumor types". In formulating this hypothesis of a mutagenic MOA, the Draft Review did not present a weight-of-evidence analysis as directed by USEPA's policies and technical guidelines. The Draft Review focused on studies conducted in bacteria (using different exposure systems) or non-mammalian species, with less weight placed on the more robust *in vitro* studies in mammalian cells and *in vivo* studies, in particular those conducted by the NTP and others. The majority of the conventional genetic toxicology studies relied upon in the Draft Review does not report positive results with administration of chloroprene. In the conduct of a weight-of-evidence analysis, the flaws and methodological limitations in the studies relied upon to draw conclusions should have been acknowledged. When these studies and their limitations are considered, along with the predominantly negative *in vitro* and *in vivo* genotoxicity tests, there is minimal evidence for mutagenicity or genotoxicity of chloroprene (NTP, 1998; Pagan, 2007). It appears that once it was concluded that chloroprene was mutagenic, that conclusion formed the basis for the hypothesized MOA in the Draft Review and the foundation for each of the steps in their MOA analyses.

The hypothesized MOA presented in the Draft Review is based on the following four major assumptions, a review of which is provided in the following section.

2.A. Critical Assumptions Used in the Draft Review to Support a Mutagenic MOA for Chloroprene

2.A.1 Chloroprene, like butadiene and isoprene, is metabolized to epoxide intermediates and both of those compounds are rodent carcinogens.

While all three compounds may be carcinogenic in rodents, the mutagenic and clastogenic profiles of butadiene and isoprene demonstrate significant differences from that of chloroprene (Tice, 1988; Tice *et al*., 1988). As shown in the **Table 10**, chloroprene does not induce effects when tested in standard *in vivo* genotoxicity screening studies in mammals. While a reactive metabolite of chloroprene, (1-chloroethenyl)oxirane, may induce mutations *in vitro* in bacterial strains (Himmelstein *et al*. 2001a), neither the administration of chloroprene (Drevon and Kuroki 1979) nor this epoxide metabolite (Himmelstein *et al*. 2001a) was genotoxic or mutagenic in *in vitro* mammalian cell gene mutation or micronucleus cell assays, respectively, in Chinese hamster V79 cells. Also, chloroprene was not genotoxic when tested *in vivo* (Tice 1988; Tice *et al*. 1988; NTP 1998; Shelby 1990; Shelby and Witt 1995). These data indicate that the reactive metabolites formed from chloroprene are effectively detoxified *in vivo* in the concentration ranges studied. This is an important and relevant difference between chloroprene and the other two compounds.

Butadiene and isoprene appear to be effective somatic cell genotoxins in mice (Tice, 1988), while chloroprene was not genotoxic in most *in vivo* assays (Tice 1988; Tice *et al*. 1988; Shelby

1990; Shelby and Witt 1995; NTP 1998). The only published chloroprene-related study showing positive chromosomal aberrations *in vivo* was a study cited by Sanotskii (1976) who reported increased chromosomal aberrations in bone marrow in mice exposed to chloroprene at concentrations of 1 ppm and lower. As previously acknowledged by USEPA, this study was technically deficient and conflicts with studies conducted by NTP in which mice were exposed to chloroprene concentrations up to 80 ppm for up to 13 weeks without evidence of chromosomal damage or sister chromatid exchanges (SCE) in bone marrow in mice (Shelby 1990; NTP 1998). Sanotskii (1976) also claimed to find increases in chromosomal damage in humans exposed to chloroprene; however, these data reported in Sanotskii (1976) also appear to be flawed because the control subject data were taken from published work and were not matched to the subjects sampled concurrent with the exposed individuals.

Two other major differences are evident from the experimental data, as noted in Sections 2.A.4 and 2.B. First the *ras* profile in lung tumors in treated animals is considerably different for chloroprene and 1,3-butadiene (Sills *et al*. 1999). The majority of the *ras* mutations in lung tumors in chloroprene-treated mice was at codon 61 (approximately 75%), while in 1,3-butadiene-treated mice the all were at codon 13 (100%) with none reported at codon 61 (Sills *et al*. 1999). This is an important distinction because codon 61 represents changes at CAA sequences, while codon 13 represents changes at CGC sequences. Secondly, the toxic effects and histopathology observed in chloroprene-treated F344 rats and B6C3F1 mice were substantially different from those seen in butadiene or isoprene exposed animals (Melnick *et al*. 1996).

In summary, the genetic toxicology data from standard *in vivo* genotoxicity tests and other data presented demonstrates that chloroprene, unlike butadiene and isoprene, does not produce measurable DNA damage or exchanges. Similar studies using cultured mammalian cells also failed to demonstrate DNA damage. The ability to produce DNA damage *in vivo* is a necessary attribute of carcinogens with a true mutagenic MOA.

2.A.2 Chloroprene is metabolized to an epoxide intermediate [(1-chloroethenyl)oxirane] which forms DNA adducts in vitro.

The principal metabolite of chloroprene is the epoxide (1-chloroethenyl)oxirane, which, when tested as a pure compound, was mutagenic in *Salmonella* strains TA100 and TA1535 (Himmelstein *et al*. 2001a). DNA sequence studies of strains TA1535 and TA100 found that they have a codon made up of –G-G-G- at the mutant site indicating that any chemical inducing reversion in these strains must be specific for either guanine (G) or cytosine (C) bases (Koch *et al*., 1994). The Koch *et al*. study suggests that (1-chloroethenyl)oxirane preferentially reacts with GC base sites and predict that DNA adducts produced by chloroprene (at least in bacteria), if present, would primarily involve guanine or cytosine bases rather than adenine (A) or thymine (T) bases. This is a key point when considering the *ras* mutation data discussed below (2.A.4).

In vitro investigations of (1-chloroethenyl)oxirane interactions with DNA were conducted by Munter *et al*. (2002; 2007a, b). In these studies, the mutagenic metabolite (1-chloroethenyl)oxirane was reacted with either pure nucleosides or with double stranded calf thymus DNA in *in vitro* cell-free systems and the DNA reaction products identified and

quantified. The two most common adducts identified involved guanine (96%) and cytosine (2%) on a picomole per milligram (pmol/mg) DNA basis. Adducts with thymine and adenine were relatively rare (in the range of 0.01%). The guanine/cytosine adducts formed by (1-chloroethenyl)oxirane would be expected to produce GC to AT transitions or GC to TA transversions and revert strains TA100 and TA1535. The results confirmed the presumption that the major epoxide metabolite of chloroprene would preferentially produce adducts at GC sites.

Studies of the DNA interactions produced by (1-chloroethenyl)oxirane are consistent with its activity in the Ames test, as reported by Himmelstein *et al*. (2001a) (as noted in section 2.A.3), but do not suggest that this metabolite would be responsible for the induction of *in vivo* mutations involving substitutions at AT sites or mutations in mammalian cells *in vitro*, as noted by the lack of genotoxicity in the study by Himmelstein *et al*. (2001a). Studies were not located in bacterial strains that respond to specific mutations at AT sites to corroborate this hypothesis.

2.A.3 Chloroprene is a point mutagen *in vitro* (in some but not all bacterial assays).

Reviews of chloroprene question the classification of chloroprene as a mutagen (NTP, 1998; Pagan, 2007) citing issues surrounding the findings in the Ames tests. **Table 11** summarizes studies of chloroprene in the Ames test.

The results of the bacterial mutagenicity studies are equivocal, at best. Two studies showed that chloroprene was mutagenic in *Salmonella typhimurium* TA100 and/or TA1535, particularly with the addition of S9 mix (Bartsch *et al*. 1979; Willems 1980). The other two studies failed to show any increase in TA1535 or TA100 revertants. Chloroprene was not mutagenic in *S. typhimurium* strains TA98 or TA1537 (Zeiger *et al*. 1987). Because toxicity to the Salmonella cells was reported for all of the studies listed in **Table 11**, one can assume there was adequate exposure to chloroprene, its metabolites or oxidative degradation products although concentrations and composition verification were not performed.

Reviewing the methods of these studies shows a clear dependence on exposure methods and the form of chloroprene (e.g., newly distilled or aged) tested. Chloroprene was only positive in studies employing a procedure in which open dishes containing the cells and overlay agar were sealed in desiccators containing chloroprene gas; whereas, a pre-incubation method which adds chloroprene to sealed vessels containing the test organisms in buffer or S9 mix before plating in overlay agar showed no evidence of mutagenicity. Both of these methods have been reported (Kier *et al*., 1986) to detect activity with volatile chemicals (e.g., vinyl chloride, dimethylnitrosamine, fluroxene).

Westphal *et al*. (1994) demonstrated the importance of both vehicle and decomposition products on the mutagenicity of chloroprene. Westphal *et al*. (1994) showed that freshly distilled chloroprene was not mutagenic in *S. typhimurium* TA100 but chloroprene aged for as little as 2 to 3 days at room temperature was mutagenic in the TA100 strain. Mutagenicity increased linearly with the age of the distillate likely due to the presence of decomposition products such as cyclic dimers (Westphal *et al*. 1994).

It is not possible to unequivocally conclude from published data that chloroprene is a point mutagen in bacteria. If chloroprene is a point mutagen in bacteria, that activity has not extrapolated to mammalian cells. Drevon and Kuroki (1979) were not able to induce point mutations when chloroprene was tested in Chinese hamster V79 cells. In their study, V79 cells were exposed in desiccators containing chloroprene for 20-30 minutes both with and without S9. Chloroprene, even at cytotoxic concentrations, did not produce increased mutation for either 8-azaguanine or ouabain resistance.

Himmelstein *et al*. (2001a) tested the primary metabolite of chloroprene, (1-chloroethenyl) oxirane, and found it to be mutagenic in the absence of S9, suggesting that this metabolite may be the reactive agent in the Ames test; however, this epoxide metabolite was not genotoxic in mammalian cells *in vitro* (Chinese hamster V79 cells) (Himmelstein *et al*. 2001a). As noted below, *in vitro* systems do not have the normal levels of detoxifying pathways found in intact mammalian cells to further metabolize/detoxify this primary metabolite.

The Ames mutagenicity profiles identified in **Table 11** raise doubts about the ability of a chloroprene metabolite to induce mutagenic changes at tumor target sites in rodents and challenges the assumption that the chloroprene MOA is through mutation. No convincing mutagenicity weight-of-the-evidence argument can be made from the available bacterial mutagenicity data. In the case of chloroprene, the results in bacterial systems were dependent on both the exposure method and the form of the chloroprene administrated. While the major epoxide metabolite did produce positive results in the *S. typhimurium* strains tested, it was not genotoxic when administered in a micronucleus test in mammalian Chinese hamster V79 cells and neither was chloroprene. It is important to note that results from the Ames test may not be an accurate predictor of carcinogenicity of chloroprene because glutathione and other detoxification pathways that would mitigate or eliminate the production of potentially active metabolites are not present in S9 microsome preparations at levels present in intact cells. Supporting this assumption was the finding that addition of glutathione to the chloroprene/metabolite Ames tests significantly diminished the reported mutagenic activity (Westphal *et al*., 1994). The absence of genotoxicity in intact mammalian cells systems and *in vivo* studies suggest that the bacterial mutagenicity data has limited significance to a genotoxicity weight-of-evidence.

Conflicting results were obtained from two studies conducted in Drosophila (sex linked recessive lethal assay, SLRL). A study reported by Vogel (1979) showed a positive response following a feeding exposure when data from various trials were pooled (none of the individual trials achieved statistical significance). A second study by Foureman *et al*. (1994) exposed Drosophila to chloroprene by direct injection of the chloroprene into the flies but failed to find an increase in mutant offspring. Feeding exposure, used by Vogel, involves maintaining the test material in the Drosophila food for many hours, which may lead to the formation of chloroprene decomposition products. Foureman *et al*. provided a discussion of the reasons for the differences with those reported by Vogel *et al*., including the likely presence of decomposition products, the strain tested, the statistical methods used and the sample size (NTP 1998; Pagan 2007). Drosophila SLRL results may not be relied upon as proof of point mutation as this test also responds to small chromosome deletions as well as point mutations (USEPA, 1988 Health Effects Test Guidelines OPPTS 870.5275).

2.A.4 The Draft Review maintains that Chloroprene is a point mutagen in vivo (in carcinogenicity bioassays with mutations identified in proto-oncogenes). Base changes identified as A to T transversions (CAA to CTA) were the primary mutations identified in proto-oncogenes from tumors of lung, Harderian gland and forestomach of mice.

Sills *et al*., (1999, 2001) produced a proto-oncogene mutation profile for some target tumors in the mouse. The *ras* family of genes may be activated by mutations in codons 12, 13 and 61. Investigators study mutations in tumors at target sites to identify "mutagen finger prints" for specific chemicals. A comparison of isoprene, chloroprene and butadiene is shown in **Table 12** .

The profile in the forestomach shows that chloroprene *ras* mutations, unlike 1,3-butadiene and isoprene, differed from control animal *ras* mutations only with respect to A to T changes. Activated *ras* mutations (at any location) in the forestomach were 4/11 in control animals (based on the incidence in a number of studies) and 4/7 in the chloroprene treated animals (Sills *et al*., 2001). Codon 12 (GGT) and codon 13 (CGC) K-*ras* mutations were found in both control (4/4) and chloroprene tumors (3/4). Only the A to T transversions in codon 61 (CAA) set chloroprene tumors apart from the control spectrum in the forestomach. Given the small numbers, these data do not provide convincing support for exogenous mutation induction. Although forestomach tumors were increased in mice, the increases were not statistically significant (NTP 1998) and the tumors could represent a promotion of spontaneous events.

Among the lung tumors, a majority (22/37) of the K-*ras* mutations were A to T transversions in codon 61 (CAA). A similar pattern was seen with Harderian Gland tumors as well (Sills *et al*., 1999). These studies established a clear specificity for A to T transversions, which does not fit the expected site-specificity defined by the (1-chloroethenyl)oxirane results in the Ames study or the DNA adduct data (Himmelstein *et al*. 2001a; Munter *et al*. 2002). Although codon 61 A to T tranversions were not observed in the control group of this study, they have been observed in mice from other tissues (Dragani *et al.* 1991).

The data provided in the report by Sills *et al*. (1999) appears to present a dilemma for the hypothesized mutation induction MOA. The number of lung tumors shows a clear dose-dependent increase across the three treatment groups but the number of A to T *ras* mutations decreases with dose. Such a relationship is not typical of mutagenic responses. One might expect to see a plateau in lung tumors, but not an increase if the underlying MOA is a K-*ras* mutation. The increases in lung tumors are more consistent with a secondary MOA (e.g., one involving cytotoxicity) at target sites, i.e., one that follows the dose-dependent tumor response (e.g., cytotoxicity induced bronchiolar hyperplasia).

An alternative explanation for the inconsistency seen between adduct specificity and proto-oncogene specificity might be that chloroprene selects for pre-existing spontaneous mutations, the prevalence of which may be species-specific and vary with the target tissue. For example, investigations of nitrosomethyl urea (NMU) rodent carcinogenesis indicated that increased H-*ras* codon 12 mutations in rat mammary tumors were due the compound acting as a promoter of spontaneous codon 12 mutations and not from the initiation of new mutations (Buzard, 1996).

2.B Alternative MOA Hypothesis

It appears that once it was hypothesized that chloroprene was mutagenic, the Draft Review did not consider other possible MOAs, as is discussed in the conduct of a weight-on-evidence analysis (USEPA 2005). Further, consideration of alternative MOAs is part of the application of the Human Relevance Framework (HRF) (Cohen *et al*. 2003; Meek *et al*. 2003; Cohen 2004; IPCS 2005; Boobis *et al*, 2006) that provides a framework by which to conduct a weight-of-evidence evaluation.

One of the arguments used in the Draft Review supporting a mutagenic MOA was the apparent similarity of chloroprene to butadiene and isoprene with respect to genotoxicity and mutations found in proto-oncogenes isolated from tumors in mice. Actually, a more detailed comparison of the attributes of butadiene, isoprene and chloroprene indicates that chloroprene is more unlike than similar to butadiene and isoprene.

The specificity of DNA adduct formation at C and G bases *in vitro* raises questions about the true origin of the A to T transversions found in forestomach and lung tissue of chloroprene-treated mice in the carcinogenicity study, especially because the forestomach tumors were not significantly increased in mice. The actual number of such A to T transversions in forestomach tumors was very small; 0/11 in the control tumors and only 2/7 in tumors from the chloroprene treated group (Sills *et al*., 2001). The small number could be explained by selection of pre-existing spontaneous mutations through cytotoxicity-induced cell proliferation (Buzard, 1996). The number of lung tumors in chloroprene-treated mice showing A to T transversions at codon 61 was higher than the incidence in the forestomach (25/46 across all dose groups) (Sills *et al*. 1999), but could be associated with toxicity to lung tissues from the relatively more toxic chloroprene. In contrast, the majority of the *ras* mutations in lung tumors in 1.3-butadiene-treated mice were at codon 13 with none reported at codon 61 (Sills *et al*. 1999).

An alternative MOA that was not explored is the possibility of a direct cytotoxic MOA. There is some experimental data supporting this MOA. For example, chloroprene-induced toxicity *in vivo* was greater than that seen by either 1,3-butadiene or isoprene following inhalation exposure (Shelby, 1990) supporting a hypothesized MOA based on target site cytotoxicity. In mice, histopathology evaluations of chloroprene target tissue are consistent with a non-genotoxicity MOA. For example, the incidence of chloroprene-induced bronchiolar hyperplasia in the respiratory system follows the increased incidence of lung tumors, whereas the incidence of lung K-*ras* mutations does not.

Melnick *et al*. (1996) reported that the toxic effects and histopathology observed in chloroprene-treated F344 rats and B6C3F1 mice were substantially different from those seen in butadiene or isoprene exposed animals. The toxic effects were specific for oral and forestomach sites. Melnick *et al*. (1996) reported chloroprene-induced hyperplasia of the forestomach in mice and degeneration and metaplasia in oral cavity of rats. These lesions were not produced by either butadiene or isoprene and could be related to the increased incidences of tumors at these sites.

Although a mitogenic response might be considered to explain observed hyperplasia, based upon the cytotoxicity seen in site of contact tissues, such as the oral and nasal cavities, a purely

mitogenic process may not be a likely explanation. A cytotoxicity-driven hyperplasia could also be occurring, which can result from cell death and subsequent tissue regeneration. Hyperplastic processes have been hypothesized to lead to selection of pre-existing oncogene and tumor suppressor gene mutations (Buzard, 1996). Extrapolation of a target site cytotoxicity MOA involving cell proliferation and tumor promotion to other tumor sites is consistent with the attributes of chloroprene.

2.C. Summary

A critical evaluation of the cytotoxic and genotoxic profiles of chloroprene suggests that this chemical acts through a different MOA than the carcinogens 1,3-butadiene and isoprene. Chloroprene's genotoxicity profile lacks several attributes that would provide the necessary support for a mutagenic MOA.

- **Standard *in vivo* tests for genotoxicity are negative**: Chloroprene, unlike butadiene and isoprene, does not exert genetic toxicity to somatic cells *in vivo*. In addition, neither chloroprene nor its major epoxide metabolite was genotoxic in mammalian cells treated *in vitro*. In order to have confidence in a mutagenic MOA, one should expect the candidate chemical to produce evidence of genotoxicity in mammalian somatic tissue(s) of the species in which it induces tumors. The results suggest that the epoxide metabolite of chloroprene is effectively detoxified during *in vivo* exposure conditions.

- **Lack of consistent data for point mutation induction**: The ability of chloroprene to produce point mutations *in vitro* (bacteria) is equivocal, at best. Chloroprene did not induce mutation in cultured mammalian cells. The conflicting specificities between *in vitro* point mutation and DNA adduct induction and *in vivo ras* mutations found in target site tumors may be of an origin other than chloroprene-induced. The *ras* "mutagen finger print" of A to T specificity needs to be reconciled with the *in vitro* mutation and DNA adduct data, which clearly shows a GC profile, in order to fully support a mutation MOA. The inconsistency in specificity raises questions as to whether the A to T mutations are a reliable indication of *in vivo* point mutation induction or are the consequence of other secondary processes.

- **A non-genotoxic MOA for chloroprene should be considered**: Some evidence exists to suggest that the reported target site specific toxicity of chloroprene in mice could be attributed to localized cytotoxicity with subsequent induction of hyperplasia and cell regeneration followed by promotion of pre-existing proto-oncogene mutations. Aspects of this alternative MOA fits the known toxic and DNA reactivity attributes of chloroprene. Butadiene and isoprene, used as comparison carcinogens, are characterized by different toxicity, genotoxicity and oncogene mutation profiles.

Chloroprene metabolites and/or breakdown products appear to be DNA reactive *in vitro*; however, extrapolation of that information directly to a mutagenic MOA is not compelling and a non-mutagenic MOA based on target site cytotoxicity should be considered.

3. Issue for Resolution: EPA Consideration of Species Differences in Toxicokinetics and Target Tissue Dosimetry.

The *Guidelines for Carcinogenic Risk Assessment* (USEPA 2005) recommend that if sufficient, relevant quantitative information is available (e.g., blood/tissue partition coefficients and pertinent physiological parameters for the species of interest that PBTK models should be constructed to assist in the determination of tissue dosimetry, species-to-species extrapolation of dose, and route-to-route extrapolation. In the case of chloroprene, all of the quantitative data necessary to refine and validate the existing peer-reviewed PBTK model for chloroprene (Himmelstein *et al*. 2004b) are now available. This PBTK model should now be applied to determine species-specific target-tissue dosimetry for incorporation into the dose-response assessment.

3.A. The discussion provided in the toxicokinetic section of the Draft Review does not adequately describe nor emphasize the critical impact that the documented species differences will have in attempting to quantify the potential toxicity and carcinogenicity of chloroprene.

The Draft Review provides a brief summary of the studies available at the time the draft was prepared that characterize, both qualitatively and quantitatively, differences across species in the toxicokinetics of chloroprene (Munter *et al*. 2003, 2007a, b; Himmelstein *et al*. 2004a, 2001a, b; Cottrell *et al*. 2001; Summer and Greim 1980; Hurst and Ali 2007). For example, data for blood/tissue partition coefficients are available in multiple animal species (i.e., mouse, F344 rat, Wistar rat, and hamster) and these are listed in Table 3-1. What is not noted or provided in the Draft Review is that blood/tissue partition coefficients are available for human tissues and that these data demonstrate a significant difference in the blood-to-air partition coefficients between animals and humans. The human blood-to-air partition coefficient is approximately one-half of the value measured in rodents, demonstrating a difference across species that is important in the determination of target tissue dose.

The available *in vitro* information on the metabolism of chloroprene (Cottrell *et al*. 2001; Himmelstein *et al*. 2001b; Himmelstein *et al*. 2004a) also demonstrates significant quantitative differences across species in the production of the major metabolites of chloroprene, and in particular, the epoxides presumed to be the active moieties. The *in vitro* studies reported that greater amounts of metabolites are produced in B6C3F1 mice and F344 rat liver microsomes, followed by the Wister rat, then in humans and hamsters. Based on data reported by Himmelstein *et al*. (2001b), the ratio of liver microsomal metabolites as a percentage of 1-butanol were 9:12:1.3 in the B6C3F1 mouse, F344 rat, and human, respectively. These differences are noted in Tables 3-2 and 3-3 of the Draft Review, but no attempt is made to consider these differences in determining the human equivalent dose to use in dose-response modeling when assessing the potential toxicity or carcinogenicity of chloroprene in humans.

Himmelstein *et al.* (2004a) also notes species differences in the detoxification of epoxide metabolites, most notably the epoxide hydrolase which serves to eliminate any epoxide formed. For example, intrinsic clearance in the liver for enzymatic hydrolysis of (1-chloroethenyl)oxirane was human ~ hamster > rat > mouse. In the lung, the order was human ~ hamster > rat ~ mouse.

Additionally, glutathione S-transferase activity was measured in liver cytosol by Himmelstein *et al*. (2004a) and for the liver the order of activity was hamster > Fischer rat ~ Wistar rat > mouse > human. As a whole, the balance of reactive metabolite formation and detoxification across species appears to indicate that the mouse would be the most sensitive species, based on higher rates of epoxide formation, slower hydrolysis, and faster GSH conjugation, with perhaps the latter leading to an imbalance in glutathione (antioxidative) status and subsequently contributing to cytotoxicity. Himmelstein *et al*. (2004a) also provide Vmax/Km values for chloroprene oxidation. These ratios suggest that hepatic intrinsic clearance was mouse ~ hamster > Fischer rat ~ Wistar rat > human. Lung intrinsic clearance was mouse >> Fischer rat ~ Wistar rat ~ hamster ~ human.

Because of the documented differences in toxicokinetics of chloroprene across species, it is critical that species-specific adjustments be made. Even in the absence of the application of a validated PBTK model, which is now available for chloroprene, chemical-specific adjustment factors (see Clewell *et al*. 2008) can be applied to derive a reasonable HEC that, while not capturing all of the toxicokinetic differences across species, would capture the difference in the key determinant of toxicity and carcinogenicity. In the case of chloroprene, the Draft Review indicates that the key determinant is metabolism to the active epoxide. At the very least, because of the potential impact of species-specific differences in target tissue dosimetry, such a discussion should have been part of the potential uncertainties in assessing the potential carcinogenicity of chloroprene. However, no chemical-specific quantitative adjustments were incorporated into the quantification of the potential risk to humans from exposure to chloroprene.

3.B. A PBTK model should have been applied to derive appropriate human equivalent doses for use in the dose-response modeling.

The availability of a published, peer-reviewed PBTK Model (Himmelstein *et al*. 2004b) is noted in the Draft Review; however, it was not used in the quantitative dose-response modeling for chloroprene. The reason provided is the need for blood or tissue time-course concentration data for model validation. It is noted in the Draft Review, however, that the published version of this model, which relies on *in vitro* metabolic information (Himmelstein *et al*. 2004a), provided satisfactory simulations of the experimental closed chamber gas uptake concentrations over a range of starting concentrations in the male mouse, Fischer rat, and hamster. However, this ability of the model to simulate the available closed chamber data for chloroprene is not recognized in the Draft Review.

In fact, the model was used to simulate internal dose in the lung and liver as amount metabolized/g tissue/day as representative of NTP bioassay exposure condition (Himmelstein *et al*. 2004b) (**Table 13**). When the internal dose for the lung was applied in a dose-response analysis of lung tumors (**Figure 9**), an improved relationship was found compared to that seen when the external exposure concentration values were used as the "dose", supporting an association between the target tissue dose estimated by the model and the observation of lung tumors in mice and rats.

Since the publication of the Himmelstein *et al*. (2004b) PBTK model, ongoing research has focused on the toxicokinetics and MOA for chloroprene. The research was designed based on the consensus of scientists at the International Symposium on the Evaluation of Butadiene &

Chloroprene Health Risks held in September, 2005, and has been supported by the International Institute of Synthetic Rubber Producers Chloroprene Scientific Oversight Committee (IISRP-SOC) on β-chloroprene. One of the studies (IISRP-12828-1388, 2009) has recently been completed and provides the necessary blood time-course concentration data that, according to the Draft Review, is needed to validate the published Himmelstein *et al*. (2004b) PBTK model. This study has been provided to the USEPA in a separate submission to Docket ID No. EPA-HQ-ORD-2009-0217 dated December 1, 2009. A summary of this research was also presented at the Listening Session for the Draft Review for Chloroprene held at the USEPA Potomac Yard Offices in Arlington, VA on November 23, 2009. A brief summary of these results is provided in Attachment B. Now that this information is available, the PBTK model should be applied in the dose-response assessment for the UR to determine a HEC.

Moreover, the metabolic parameters documented in the Himmelstein *et al*. (2004b) PBTK have been refined based on new measurements of oxidation in female rodent tissues (liver, lung, and kidney), and male rodent tissue (kidney) in a recently completed study (IISRP-17520-1388) (**Table 14**). A summary of this study was also provided to the USEPA in a separate submission to Docket ID No. EPA-HQ-ORD-2009-0217 dated December 1, 2009, with a presentation made at the Listening Session on November 23, 2009. *In vitro* microsomal metabolism time course data collected at DuPont Haskell Global Centers were sent for kinetic modeling at the Hamner Institutes. These data have been analyzed using a two-compartment *in vitro* kinetic model (Himmelstein *et al*. 2004a). Point estimates for Vmax and Km have been obtained and probability analyses completed as a key step to better define parameter variability when scaling the parameters for incorporation in the *in vivo* PBTK model. The use of the refined metabolic parameters increase the confidence and decrease uncertainty when applied to *in vivo* PBTK model simulations used in dosimetric adjustments for human exposures.

3.C. New Genomics Data

New research has recently been completed that explores more fully the potential mode-of-action for the lung tumors observed in the mouse and rat following chronic exposure to chloroprene (IISRP-12828-1389). A summary of this study was provided to the USEPA in a separate submission to Docket ID No. EPA-HQ-ORD-2009-0217 dated December 1, 2009. A summary of this research was also presented at the Listening Session for the Draft Review for Chloroprene held at the USEPA Potomac Yard Offices in Arlington, VA on November 23, 2009. In this study, gene expression microarray measurements were conducted in lung tissue from female B6C3F1 mice and F344 rats following exposures to chloroprene that both span cancer bioassay concentrations and provide approximately equal target tissue levels in the lung (i.e., total amount metabolized per gram tissue per day) calculated using the existing physiologically based toxicokinetic (PBTK) model (Himmelstein *et al*., 2004b). Phenotypic anchoring for the gene expression data was performed by evaluating cell proliferation by BrdU labeling in mice and histopathological changes in the lungs of both mice and rats.

Chloroprene exposure caused minimal hyperplasia in the terminal bronchioles of the mouse lung at the highest exposure concentration (90 ppm) after 5 or 15 days of exposure. Analysis for cell proliferation in the mouse lung using BrdU staining showed a clear dose response in the terminal bronchiole regions. Histopathological effects and increased cell proliferation were absent in the alveolar regions. In the rat, histopathology effects were entirely absent up to the highest

exposure concentration (200 ppm). ANOVA analysis of gene expression changes showed no statistically significant changes at the lowest exposure concentrations in either species (0.3 ppm for mouse or 5 ppm in rat).

Benchmark dose (BMD) analysis of the gene expression data was performed together with gene ontology (GO) classification and KEGG (Kyoto Encyclopedia of Genes and Genomes) pathways to model the transcriptional dose-response data and estimate the concentration at which different cellular processes and pathways were altered. In the mouse, temporal changes in the categories and pathways for each exposure concentration were minimal with the 5- and 15-day time points giving approximately the same BMD values for the endpoints evaluated (**Figure 8**). Using exposure concentration, changes in gene expression related to glutathione biosynthesis began around 0.9 ppm while changes in gene expression related to DNA damage occurred around 2.5 ppm and cell proliferation around 14 ppm. The sequential activation of these pathways was dose related. Activation of these responses was also consistent with both the cell proliferation data and the tumor response data where mouse lung tumors were observed at the lowest dose tested in the bioassay (12.8 ppm). In the rat, temporal changes in the key functions were more apparent with the 15-day time point producing consistently higher BMD values in the rat than the mouse. Using exposure concentration, gene expression changes related to glutathione biosynthesis, DNA damage, and cell proliferation in the rat all occurred between 20 and 25 ppm at the 15-day time point. When normalized to preliminary measures of internal dose based on the PBTK modeling effort (IISRP-17520-1389), the gene expression changes in the rat for the key categories at the 15-day time point were highly consistent with those observed in the mouse, but occurred at comparatively higher chloroprene exposure concentrations. These results lend support to the hypothesis that the observed differences in tumor response in the chronic animal bioassays may be related not only to species-specific differences in sensitivity, but also to species-specific differences in metabolism.

3.D Summary

The incorporation of species-specific dosimetric values into the dose-response assessment for chloroprene will have a direct impact on the proposed UR. Application of the refined PBTK model is being conducted to predict internal doses in known target tissues of rodents and in turn estimate HECs using a human version of the PBTK model (IISRP-17520-1389). A summary of this study was provided to the USEPA in a separate submission to Docket ID No. EPA-HQ-ORD-2009-0217 dated December 1, 2009. A summary of this research was also presented at the Listening Session for the Draft Review held at the USEPA Potomac Yard Offices in Arlington, VA on November 23, 2009. Comparison with reported exposure modeling (Marsh *et al*. 2007) from a recent assessment done for the human epidemiological work is also planned. This approach has been successfully used for other volatile chemicals (e.g., vinyl chloride, Clewell *et al*., 2001) and is being applied here to improve the quantitative risk assessment for chloroprene. A significant advancement will be the application of stochastic (probability) analysis of the key model parameters and the evaluation of these as they relate to potential human exposure. The results of this analysis will be critical to the quantitative interpretation of the existing toxicological database for chloroprene, because it will put in perspective the non-positive epidemiological data with the positive rodent bioassay results by cross-species comparison of target tissue dosimetry in both the mouse and the human. The consideration of the new research

providing validation of the PBTK model is highly relevant to interpreting the available toxicological data for chloroprene and quantifying the potential impact on human health.

New genomics information provides evidence of differences in response across species (mice and rats) that reflects more than just kinetic differences in the production and retention of reactive metabolites. The gene expression changes observed in the rat in the recently completed genomics study (IISRP-12828-1389) occurred at comparatively higher chloroprene exposure concentrations than those observed in the mouse. When exposure was normalized to preliminary measures of internal dose, based on the PBTK model, they were highly consistent with those observed in the mouse. These results lend support to the hypothesis that the observed differences in tumor response in the chronic animal bioassays are related to species-specific differences in metabolism.

In summary, the results of this new research support the use of a PBTK model to estimate dosimetric values to quantify species differences in chloroprene toxicokinetics. The recently completed toxicokinetic studies provide measured time course blood data to support validation of the PBTK model. Now that this model is available, the use of tissue-specific dosimetry to derive the HEC can be justified to use in the quantitative dose-response analysis of the rodent bioassay data. The ability to account for species differences in toxicokinetics is critical to the analyses in the Draft Review.

4. Issue for Resolution: USEPA Selection of Critical Decision Points in the Determination of the RfC and UR.

In the absence of positive epidemiological evidence that could be used quantitatively, the use of the NTP (1998) study as the principal study to estimate the RfC and UR in the Draft Review is appropriate, as this study provides excellent dose-response information for both non-cancer and cancer endpoints. However, a number of issues were identified regarding the modeling and treatment of these data. Improvements and recommendations are summarized below.

4.A. Decision Points in the Determination of the RfC

The presentation of the potential data sets presented in the Draft Review to be used to determine the RfC (page 5-3, Table 5-1), including the data set ultimately selected for the RfC (*i.e.*, nasal lesions in male rats) needs additional information. For example, Table 5-1 is potentially misleading, in that it suggests by omission that nasal effects are only observed in male rats. Table entries for nasal effects in female rats are listed as "not observed", which is incorrect. Atrophy and necrosis were observed in the nasal olfactory epithelium of female rats as well (NTP 1998). Also missing from Table 5-1 in the Draft Review are the data for nasal atrophy in male and female mice (NTP 1998). These data should be included because the identification of nasal lesions in rats and mice of both sexes increases the confidence in using this endpoint as the basis for the RfC.

The calculations for determining an adjusted Point of Departure (POD_{adj}) for chloroprene (page 5-4, line 24) includes the default assumption that the nasal effects are attributable to the cumulative exposure to chloroprene (6 hours/24 hours * 5 days/7 days = 0.18). However, some

uncertainty remains regarding whether these effects are attributable to peak exposure rather than cumulative exposure, in which case the duration adjustments would not be necessary. Although data are lacking to move away from the default assumption, peak concentrations, rather than a daily average concentration, have been relied upon in the derivation of an RfC or RfD. An example is in the determination of an RfC and RfD for ethylene glycol butyl ether (EGBE) reported in the Draft Toxicological Review for EGBE, which relied upon peak concentration, rather than a daily average (USEPA 2008). Therefore, in the case of the endpoint relied upon for the derivation of the RfC, peak concentration should be considered as a potential dose metric along with average cumulative exposure.

The Point of Departure HEC for the derivation of the RfC is a Benchmark Concentration of 1.0 mg/m^3, based on the incidence of atrophy and necrosis in the olfactory tissue of the male rat upper respiratory tract. USEPA presents the calculations for determining a HEC at the point of departure (Page 5-5, lines 19-29). Specifically, a Regional Gas Dose Ratio (RGDR) of 0.28 is calculated to account for species differences in extrathoracic tissue dosimetry, based upon methods described in USEPA (1994) for a Category 1 gas. This approach is inconsistent with current science on dosimetry. In USEPA's recent review of the science regarding upper respiratory tract (URT) dosimetry (USEPA, 2009), it was concluded that,

> "…*for gas deposition in the URT the internal target tissue dose equivalency between humans and rats is achieved through equivalency at the level of the externally applied concentration, i.e., for both rats and humans, the same external air concentration leads to the similar internal target-site dose to the URT."*

Based upon these recommendations, the application of the adjustment of 0.28 is not necessary.

A value of 3 for database deficiencies (UF_d) for chloroprene (page 5-8, lines 1-10) is incorporated in the derivation of the RfC. However, several lines of evidence suggest that this value may not be needed. First, chloroprene is not expected to accumulate in tissues such that in a multigenerational study, exposures to the second generation (F2) would be greater than experienced by the first generation (F1). Second, the results of a single generation reproductive toxicity study for a structurally similar chemical, 2,3-dichloro-1,3-butadiene (Mylchreest *et al*. 2006), indicate that effects at the point of contact (i.e., nasal effects) in parental animals are more sensitive than reproductive/developmental effects. Specifically, this study reported a No Observed Adverse Effect Level (NOAEL) of 10 ppm for nasal effects in rats, and a NOAEL of 50 ppm for reproductive toxicity. Changes in maternal and fetal body weights were noted at 50 ppm in the developmental portion of the study. Similarly, an unpublished one-generation reproductive toxicity study of chloroprene in rats reported a NOAEL of 100 ppm for reproductive toxicity (Appelman and Dreef van der Meulan 1979). Based on this comparison of NOAELs, USEPA should reconsider the application of an UF for database deficiencies for the lack of a two-generation reproductive study.

4.B. Decision Points in the Determination of the UR

USEPA has decided to use a Multistage-Weibull time-to-tumor dose-response model to estimate the cancer potency of chloroprene, due to increased mortality observed in the key study (page 5-15, lines 10-24). Use of this model is problematic for a number of reasons, as discussed below.

In the Draft Review, a proprietary software program (TOX_RISK version 5.3) is relied upon for the time-to-tumor dose-response modeling. This software is no longer available to the general public. As such, the transparency the dose-response assessment, one of the four principles of USEPA's *Risk Characterization Handbook* (USEPA 2000), is adversely affected. While the model applied is appropriate because a time-to-tumor model is needed, similar modeling capabilities are needed in the USEPA's publically available dose-response modeling program, BMDS. This will increase the ability of reviewers of the dose-response assessment to reproduce the results provided in the Draft Review and make them more transparent.

Use of a time-to-tumor model can be useful when early mortality results in animals dying prior to the development of tumors (*i.e.*, thereby potentially underestimating cancer potency). The effects of chloroprene on mean survival time (relative to controls) at the highest tested concentration (80 ppm) are relatively modest in female mice (~18%) and much smaller in male mice and rats of both sexes (2 to 6%) (**Figure 10**). Because an appropriate time-to-tumor model such as that provided in Tox_Risk is not readily available to the public as is BMDS, the application of the simpler models provided in BMDS (i.e., Multistage, Log-Logistic) should be applied as an alternative to examine whether the application of the more complicated time-to-tumor model has a significant impact on the estimation of the UR.

In implementing the time-to-tumor model as reported in the Draft Review, it was assumed that hemangiosarcomas were fatal, while all other tumors were incidental. This assumption does not appear to be consistent with the data. USEPA assumed that the circulatory tumors are the only lethal tumors contributing to early mortality (i.e., all others are incidental), and as such could impact the incidence of late-appearing tumors. If this were the case, one would expect to see a consistent dose-response trend for circulatory tumors with each increase in concentration. However, the dose-response data for circulatory tumors are clearly non-monotonic, showing a decreased incidence at the high concentration (when compared to the mid concentration), while the incidence for all other tumor types of interest (e.g., lung) all increase monotonically (**Figure 11**). This pattern is more consistent with early mortality from other tumor types affecting circulatory tumor incidence at the high concentration. Furthermore, inspection of average day of death with tumor in female mice (all treatment groups combined) for all eight types of interest also suggests a potential role for other tumor types being lethal: mammary gland < Zymbal's gland < circulatory < skin < lung < Harderian gland < liver < forestomach. Because there is uncertainty in any lethality assumptions made, if USEPA chooses to continue to rely upon a time-to-tumor model (not recommended based upon other concerns), some examination of the impact of viable alternative lethality assumptions appears to be warranted.

The method for selection of the model for the time-to-tumor analysis was not well characterized, as the probability associated with the χ^2 value used as a critical value for comparing the difference between two log-likelihoods was not provided. Since the Akaike Information Critera

AIC, a measure of the goodness of fit of a statistical model to a dataset, was used as the criterion for the choice of model in the RfC, it could have also been used for the assessment of the time-to-tumor modeling. In either case, the goodness of fit results for all of the models applied to the data along with the goodness-of-fit criteria for each should have been provided.

USEPA states that by modeling each of the tumor sites separately, they have assumed that the tumors are statistically independent (page 5-20, lines 4-10). While statistical independence may be assumed, because it is assumed that the MOA for the development of all tumors is the same and dependent upon the generation of the same metabolites, mechanistic or biological independence is not established. Therefore, the summing of URs is overstating the potential carcinogenicity of chloroprene, especially since individual animal data from the key study (NTP 1998) demonstrate considerable overlap in tumor incidence data (Figure 12). The standard practice of the estimation of the UR associated with the most sensitive endpoint in the most sensitive species should be protective of the other responses observed

The calculation of an overall UR for chloroprene based on summing URs from multiple separate tumor types has no precedence in IRIS Toxicological Reviews that are final and results in difficulties in interpretation. The difficulties with this method for combining the URs are based on the following observations.

- As demonstrated with Figure 12, the assumption of independence of the tumors is not appropriate in this case.
- It is assumed that the estimates of the URs are normally distributed around the MLE with, for example, the 95% UCL for the UR being equal to the MLE (mean) plus 1.645 times the standard error. This is incorrect for several reasons:
    a. The estimation (model-fitting) procedure is the BMD that is estimated and the BMDL is the 95% lower confidence limit for the BMD. That does not imply that the UR (defined as 0.1/BMDL) is the 95% UCL for the ratio 0.1/BMD.
    b. As seen by the definition of the UR, it is a ratio of a fixed value divided by an estimate of a parameter (the concentration associated with 0.1 extra risk). There is no reason to think that such a ratio would be normally distributed around a mean value even if BMD estimates were normally distributed around a mean (MLE). Considering that the ratio is constrained to be positive since the BMD and BMDL by definition must be positive, it is clear that the assumption of normality is inappropriate.
    c. The estimation of the BMDL in software such as BMDS or TOX_RISK v 5.3 does *not* make such simplistic assumptions (e.g., that the BMD estimates are normally distributed about an MLE); it uses a profile likelihood procedure that identifies the likelihood of various BMD values and selects the smallest value that gives a likelihood that could not be rejected with 95% confidence.
    d. To state that this is a statistically based approach is therefore wrong as the underlying assumption of the analysis (i.e. normality of the UR estimates) has not been shown to be true and, based on the type of data, that assumption is inappropriate.

The points of departure for each tumor type are identified in the Draft Review in Table 5-7 for female mice. Reported values for the $BMDL_{10}$ range from $3.38x10^3$ to $7.86x10^4$ $\mu g/m^3$, which corresponds to approximately 0.9 to 22 ppm. The points of departure for two tumor types (lung and liver) appear to fall considerably below the range of observation (*i.e.,* by more than a factor of 3), and therefore are inconsistent with USEPA (2000) guidelines for benchmark dose modeling, which state,

> *"The major aim of benchmark dose modeling is to model the dose-response data for an adverse effect in the observable range (i.e., across the range of doses for which toxicity studies have reasonable power to detect effects) and then select a "benchmark dose" at the low end of the observable range to use as a "point of departure" for deriving quantitative estimates below the range of observation and to use as a basis for comparison of effective doses corresponding to a common response level across chemicals or endpoints."*

Therefore, careful consideration of these results should be applied and provide additional rational for not summing results of these analyses.

Finally, the lung tumor response data was assessed in the Draft Review assuming the responses were either portal-of-entry effects or systemic effects (page 5-16, lines 24-26). This approach is internally inconsistent with the non-cancer assessment in which the nasal atrophy/necrosis and lung hyperplasia in rodents are attributed as portal-of-entry effects.

4.C Summary

Based upon these considerations, the most appropriate approach for derivation of an UR for chloroprene is to rely upon the most sensitive tumor endpoint (i.e., lung tumors) in the most sensitive species, considering relevance to human health, and proceed to use a time-to-tumor model to address early mortality associated with the less sensitive tumor types. In addition, dosimetry adjustments have been applied in the derivation of the RfC and similar adjustments are needed to calculate the HEC values for each tumor type (page 5-16, lines 17-33). Because the UESPA's proposed MOA for chloroprene may include the formation of an epoxide metabolite, it is important that the HEC values take into consideration important species differences in metabolism. This is best accomplished within the framework of a PBTK model. A PBTK model is available for chloroprene (Himmelstein *et al*. 2004b) and has been refined and validated per recently collected data (see Section 3).

5. Issue for Resolution. USEPA's Quality Control in Reporting of Chloroprene Data

In comparing information provided in the Draft Review to that in the primary literature, numerous inconsistencies were noted. In addition, information on the production of chloroprene noted in the Draft Review is not current and there are issues in attempting to duplicate some of the quantitative analyses. Attachment C provides a list of these inconsistencies by section and topic of the Draft Review Document. The contributors of this Comment document respectively request that these items be addressed prior to finalization of the Draft Review.

Summary and Conclusions

There are several major issues in the interpretation of the available toxicological data for chloroprene in the Draft Review that warrant change. With regard to the epidemiological data, there is no compelling evidence for an increased risk of mortality from total cancer or lung or liver cancer when the available studies are evaluated using a comprehensive weight-of-evidence approach. Although animal studies provide a positive response for carcinogenicity, species-specific differences in response to chloroprene exposure are observed. These likely reflect quantitative differences in toxicokinetics across species, specifically related to differences in both species differences in sensitivity and in metabolism and detoxification of potentially active metabolites. In the current Draft Review, no attempt was made to quantitatively account for these differences between the mouse, rat, and human. When genotoxicity/genomics, mode of action and toxicokinetic data are considered in an integrated manner, these data strongly suggest that the responses from chloroprene are largely confined or unique to the mouse. Because of these differences, use of the mouse data, in the absence of positive epidemiological data that can be used quantitatively, must incorporate tissue-specific dosimetry and metabolic differences.

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TABLES

Table 1. Quality Rankings for Eight Cohort Studies Investigating the Carcinogenicity of Occupational Chloroprene Exposure

EPA Criteria	Cohort Location							
	Kentucky[1]	N. Ireland[1]	Louisiana[1]	France-M[*1]	Armenia[2]	France-I[†3]	Russia[4]	China[5]
Clear objectives	H‡	H	H	H	H	H-M	H	M
Comparison groups	H	H-M	H-M	M	M	M	M-L	L
Exposure	H	H	H	H	M	M	L	L
Follow-up	H	H-M	H	H-M	M-L	M-L	M-L	M-L
Case ascertainment	H	H-M	H-M	H-M	M	M	M	H-M
Control of bias	H-M	H-M	H-M	M	M-L	M	M	M-L
Sample size	H	H	M	L	M-L	L	H-M	M-L
Data collection and evaluation	H	H	H	H	M	M	M-L	M-L
Adequate response	H	H	H	H	M	M	M	H-M
Documentation of results	H	H	H	H	M-L	M	M	L
Overall rank (1=best)	1	2	3	4	5	5	5	6

from Bukowski, 2009

* M=Mortality
† I=incidence
‡ Subjective estimate of study quality for each specific criterion H=high, M=medium, L=low
1 – Marsh *et al* 2007
2 – Bulbulyan *et al* 1999
3 – Colona *et al* 2001
4 – Bulbulyan *et al* 1998
5 – Li *et al* 1989

Table 2. Relative Size of Marsh *et al.* (2007a, b) Study Compared with Other Available Studies

Study	Subjects (Person-years)	Lung Cancer Deaths	Liver Cancer Deaths
Bulbulyan *et al*. 1998	5185 (70,328)	31	10
Bulbulyan *et al*. 1999	2314 (21,107)	3	3
Colonna and Laydevant 2001	717 (17,057)	9	1
Leet and Selevan, 1982	n/a	n/a	n/a
Li *et al*. 1989	1258 (20,105)[a.]	2	6
Total Other Studies	**9474 (128,597)**	**45**	**20**
Marsh *et al*., 2007a (L)	5507 (197,010)	266	17
Marsh *et al*., 2007a (M)	4849 (127,036)	48	1
Marsh *et al*., 2007a (P)	1357 (30,660)	12	0
Marsh *et al*., 2007a (G)	717 (17,057)	10	1
Total Marsh *et al.* (2007a, b) Study	**12,430 (372,672)**	**336**	**19**
Combined Studies	**21,904 (501,269)**	**381**	**39**
Marsh *et al.* (2007a, b) / Combined Studies	**57 % (74%)**	**88%**	**49%**

a. Taken from Bukowski (2009)

Table 3. Comparison of Reported and Estimated 95% Confidence Intervals by Bulbulyan *et al.* (1999).

		Reported 95% Confidence Limits		Recalculated 95% Confidence Limits *	
OUTCOME	**SMR or SIR**	**Lower**	**Upper**	**Lower**	**Upper**
LIVER CANCER					
Cohort SMR	3.39	1.09	10.5	0.70	9.91
Cohort SIR	3.27	1.47	7.27	1.20	7.12
Duration of Employment SIR					
< 1 year	--			--	--
1 - 9 years	3.69	0.52	26.2	0.09	20.56
10 – 19 years	2.91	0.41	20.7	0.07	16.21
20 + years	3.45	1.29	9.20	0.94	8.83
Duration of CP Exposure SIR					
< 1 year	--			--	--
1 – 9 years	1.90	0.27	13.5	0.05	10.59
10+ years	4.56	1.90	11.0	1.48	10.64
Duration of High CP Exposure SIR					
< 1 year	1.46	0.21	10.4	0.04	8.13
1 – 9 years	2.00	0.28	14.2	0.05	11.14
10+ years	6.12	2.30	16.3	1.67	15.67
Cumulative CP Exposure SIR					
1 – 14 unit-years	--			--	--
15 - 39 unit-years	2.93	0.41	20.8	0.07	16.32
40+ unit-years	4.86	2.02	11.7	1.58	11.34
LUNG CANCER					
Cohort SMR	0.50	0.16	1.55	0.10	1.46
Cohort SIR	0.53	0.24	1.19	0.19	1.15

** 95% CI recalculated using Breslow and Day (1987) method.*

Table 4. Comparison of Reported Observed Liver Cancer Cases, Expected Counts, and Standardized Ratio Estimates with 95% Confidence Intervals for Cohort Studies of Chloroprene-Exposed Workers.

STUDY	OUTCOME	OBSERVED	EXPECTED*	SMR or SIR	95% Confidence Limits		p-value
					Lower	Upper	
Li *et al*. 1989							
	Cohort SMR	6	2.48	2.42	0.89	5.27	0.08
	Monomer Workshop SMR	4	0.83	4.82	1.31	12.34	0.02
	Monomer Operator SMR	2	0.43	4.65	0.56	16.80	0.14
	Maintenance Mechanic SMR	2	0.12	16.67	2.02	60.22	0.01
	Polymer Workshop SMR	2	1.21	1.65	0.20	5.96	0.68
	Polymer Mechanic SMR	2	0.56	3.57	0.43	12.90	0.22
Bulbulyan *et al*., 1998							
	Cohort SMR	10	4.17	2.40	1.15	4.41	0.02
	Female workers SMR	8	3.48	2.30	0.99	4.53	0.05
	Male workers SMR	2	0.83	2.40	0.29	8.67	0.41
Bulbulyan *et al*., 1999**							
	Cohort SMR	3	0.88	3.39	0.70	9.91	0.12
	Cohort SIR	6	1.83	3.27	1.20	7.12	0.02
Duration of Employment SIR							
	< 1 year	0	0.06	--	--	--	--
	1 - 9 years	1	0.27	3.69	0.09	20.56	0.48
	10 – 19 years	1	0.34	2.91	0.07	16.21	0.58
	20 + years	4	1.16	3.45	0.94	8.83	0.06
Duration of CP Exposure SIR							
	< 1 year	0	0.21	--	--	--	--
	1 – 9 years	1	0.53	1.90	0.05	10.59	0.82
	10+ years	5	1.10	4.56	1.48	10.64	0.01

Table 4. Comparison of Reported Observed Liver Cancer Cases, Expected Counts, and Standardized Ratio Estimates with 95% Confidence Intervals for Cohort Studies of Chloroprene-Exposed Workers. (continued)

STUDY	OUTCOME	OBSERVED	EXPECTED*	SMR or SIR	95% Confidence Limits		p-value
					Lower	Upper	
Bulbulyan *et al*., 1999**							
Duration of High CP Exposure SIR							
	< 1 year	1	0.68	1.46	0.04	8.13	0.99
	1 – 9 years	1	0.50	2.00	0.05	11.14	0.79
	10+ years	4	0.65	6.12	1.67	15.67	0.01
Cumulative CP Exposure SIR							
	1 – 14 unit-years	0	0.46	--	--	--	--
	15 - 39 unit-years	1	0.34	2.93	0.07	16.32	0.58
	40+ unit-years	5	1.03	4.86	1.58	11.34	0.01
Colonna and Laydevant, 2001							
	Cohort SIR	1	0.73	1.36	0.03	7.63	1.00
Period of 1st Employment SIR							
	Before 1977	1	0.61	1.64	0.04	9.14	0.91
	After 1977	0	0.12	--	--	--	--
Duration of CP Exposure SIR							
	< = 10 years	0	0.15	--	--	--	--
	11 – 20 years	0	0.31	--	--	--	--
	> 20 years***	1	0.27	3.70	0.09	20.64	0.47
CP Exposure Level SIR							
	Low	0	0.13	--	--	--	--
	Medium***	1	0.25	4.00	0.10	22.29	0.44
	High	0	0.34	--	--	--	--

** Expected = reported estimate when available, when not reported in original study: Expected = Observed / SMR*

*** 95% CI recalculated using Breslow and Day (1987) method. See Table 6 for differences with reported estimates*

**** SIR not presented in original document, the listed values represent recalculated estimates*

Table 4. Comparison of Reported Observed Liver Cancer Cases, Expected Counts, and Standardized Ratio Estimates with 95% Confidence Intervals for Cohort Studies of Chloroprene-Exposed Workers. (continued)

STUDY	OUTCOME	OBSERVED	EXPECTED*	SMR or SIR	95% Confidence Limits		p-value
					Lower	Upper	
Marsh *et al*. 2007a							
	Louisville Cohort SMR	17	16.35	1.04	0.61		
	Maydown Cohort SMR	1	4.17	0.24	0.01		
	Pontchartrain Cohort SMR	0	--	--	--	--	--
	Grenoble Cohort SMR	1	1.79	0.56	0.01		
Louisville Cohort SMR (local reference)							
	Cohort SMR	17	18.89	0.90	0.53	1.44	0.78
	White race	16	15.69	1.02	0.58	1.65	1.00
	Non-White race	1	3.13	0.32	0.01	1.77	0.36
	Male sex	16	17.98	0.89	0.51	1.45	0.75
	Female sex	1	0.94	1.06	0.03	5.93	1.00
	Blue collar pay type	17	18.28	0.93	0.54	1.49	0.89
	Short-term worker	4	8.16	0.49	0.13	1.26	0.18
	Long-term worker	13	10.74	1.21	0.64	2.07	0.57
Duration of employment							
	< 5 years	4	8.16	0.49	0.13	1.25	0.18
	5 – 19 years	6	3.57	1.68	0.62	3.66	0.30
	20+ years	7	7.14	0.98	0.40	2.03	1.00
Time since 1st employment							
	< 20 years	1	1.79	0.56	0.01	3.11	0.93
	20 – 29 years	3	3.30	0.91	0.19	2.66	1.00
	30 + years	13	13.68	0.95	0.50	1.62	1.00
CD exposure status							
	Exposed	17	18.89	0.90	0.53	1.44	0.78

** Expected = reported estimate when available, when not reported in original study: Expected = Observed / SMR*

Table 5. Comparison of Selected Observed Lung Cancer Cases, Expected Counts, and Standardized Ratio Estimates with 95% Confidence Intervals for Cohort Studies of Chloroprene-Exposed Workers.

STUDY	OUTCOME	OBSERVED	EXPECTED*	SMR or SIR	95% Confidence Limits		p-value
					Lower	Upper	
Li *et al*. 1989							
	Cohort SMR	2	0.39	5.13	0.62	18.53	0.12
	Monomer Workshop SMR	1	0.08	12.50	0.32	69.65	0.15
	Maintenance Mechanic SMR	1	0.10	10.00	0.25	55.72	0.19
Bulbulyan *et al*., 1998							
	Cohort SMR	31	22.14	1.40	0.95	1.99	0.09
	Female workers SMR	14	12.73	1.10	0.60	1.85	0.79
	Male workers SMR	17	10.00	1.70	0.99	2.72	0.05
Bulbulyan *et al*., 1999**							
	Cohort SMR	3	6.00	0.50	0.10	1.46	0.30
	Cohort SIR	6	11.32	0.53	0.19	1.15	0.13
Colonna and Laydevant, 2001							
	Cohort SIR	9	4.9	1.84	0.84	3.49	0.12
Period of 1st Employment SIR							
	Before 1977	8	4.03	1.99	0.86	3.91	0.11
	After 1977	1	0.87	3.69	0.03	6.40	1.00
Duration of CP Exposure SIR							
	< = 10 years	1	0.95	1.06	0.03	5.86	1.00
	11 – 20 years	3	2.01	1.49	0.31	4.36	0.65
	> 20 years	5	1.94	2.57	0.84	6.02	0.10
CP Exposure Level SIR							
	Low	4	0.86	4.63	1.27	11.91	0.02
	Medium	2	1.60	1.25	0.15	4.51	0.95
	High	3	2.43	1.23	0.26	3.61	0.88

Table 5. Comparison of Selected Observed Lung Cancer Cases, Expected Counts, and Standardized Ratio Estimates with 95% Confidence Intervals for Cohort Studies of Chloroprene-Exposed Workers. (continued)

STUDY	OUTCOME	OBSERVED	EXPECTED*	SMR or SIR	95% Confidence Limits		p-value
					Lower	Upper	
Marsh *et al*. 2007a							
Louisville Cohort SMR		266	354.6	0.75	0.66	0.85	<0.01
Maydown Cohort SMR		48	60.76	0.79	0.58	1.05	0.10
Pontchartrain Cohort SMR		12	19.35	0.62	0.32	1.09	0.10
Grenoble Cohort SMR		10	11.76	0.85	0.41	1.56	0.75
All plants SMR		336	448.00	0.75	0.67	0.84	<0.01

** Expected = reported estimate when available, when not reported in original study: Expected = Observed / SMR*

*** 95% CI recalculated using Breslow and Day (1987) method. See Table 6 for differences with reported estimates*

Table 6. Liver Cancer Mortality and SMRs Provided by the Investigators of Marsh *et al.* (2007a, b)

Time Since First Exposure to Chloroprene	Observed Deaths	SMR	95% CI
<30 years	4	0.79	0.22-2.22
30-39	5	0.89	0.29-2.08
40+	8	0.98	0.42-1.94

Table 7. Exposure-Response Analysis for Vinyl Chloride Exposure and Liver Cancer

Metric [a.]	Internal Rate Analysis			External Rate Analysis [b]		
	Deaths	Noncases [c.]	RR [d.,e.] (95% CI)	p-value	Pyrs [f.]	SMR (95% CI)
VC_AIE						
unexposed	15	1952	1	global = 0.46 trend = 0.20	147,518	1.07 (0.60 - 1.77)
>0 - 0.27	1	139	1.04 (0.02 - 7.04)		10,880	0.98 (0.03 - 5.48)
0.28 - 1.75	0	223	0.49[g.] (-∞,2.98)		14,543	2.59
1.751+	1	367	0.43 (0.01 - 2.92)		24,978	0.37 (0.01 - 2.04)
VC_Cum						
unexposed	15	1952	1	global = 0.54 trend =0.31	147,518	1.07 (0.60 - 1.77)
>0 – 0.4476	0	164	0.66[g.] (-∞,4.00)		14,506	3.25
0.4477 - 1.9482	1	168	0.97 (0.02 - 6.67)		11,583	0.86 (0.02 - 4.79)
1.9483+	1	397	0.38 (0.01 - 2.58)		24,312	0.36 (0.01 - 1.99)

* p<.05
** p<.01
[a.] Categories based on approximate quartiles of all cancer deaths; decimal places of cutpoints reflect precision needed for computational purposes only and not precision of exposure assessment
[b.] Local county rates
[c.] The number of persons in decedent's risk set used in calculation of RR
[d.] Analyzed using LogXact
[e.] Also adjusted for sex
[f.] The number of person-years used in calculation of SMR
[g.] Median-unbiased estimate

Table 8. Chloroprene Exposure-Response for Liver Cancer from the Louisville, Kentucky Cohort

Chloroprene in the Presence of VC			
Metric	**OBS**	**SMR[a]**	**95% CI**
AIE (ppm)			
Unexposed	15	1.07	0.60 -1 .77
>0 - 11.2396	1	0.71	0.02 - 3.97
11.2397 - 15.9999	0	--	0 - 4.61
16+	1	0.37	0.01 – 2.06
Cum (ppm - yrs)			
Unexposed	15	1.07	0.60 - 1.77
>0 - 7.39	0	--	0 - 2.69
7.40 - 42.095	1	0.81	0.02 - 4.53
42.096+	1	0.40	0.01 - 2.24
Chloroprene in the Absence of VC			
Metric	**OBS**	**SMR[a]**	**95% CI**
AIE (ppm)			
>1.9364	3	0.44	0.09 - 1.29
1.9364 - 6.9114	4	1.11	030 - 2.85
6.9115 - 15.999	7	2.26	0.91 - 4.65
16+	3	0.56	0.12 - 1.64
Cum (ppm - yrs)			
< 4.594	3	0.49	0.10 - 1.44
4.594 - 45.733	1	0.21	0.01 - 1.16
45.734 - 132.215	7	1.78	0.71 - 3.66
132.216+	6	1.43	0.53 - 3.11

[a]Local county rates

Table 9. Chloroprene Exposure-Response, Respiratory System Cancer, Louisville

Metric	OBS	RR[a]	95% CI	RR[b]	95% CI
AIE (ppm)		**(p^g=0.06)**	**(p^t=0.20)**	**(p^g=0.07)**	**(p^t=0.29)**
<3.6	56	1.00		1.00	
3.6 - 8.1	70	1.34	0.93 - 1.95	1.32	0.94 - 1.91
8.2 - 15.9	33	0.88		0.86	
16+	107	1.36	0.97 - 1.91	1.32	0.93 - 1.86
Cum (ppm - yrs)		**(p^g=0.07)**	**(p^t=0.71)**	**(p^g=0.06)**	**(p^t=0.63)**
<4.7	62	1.00		1.00	
4.7 - 55.8	67	1.00		0.99	
55.9 - 164.0	77	1.32	0.94 - 1.88	1.32	0.93 - 1.88
164.1+	60	0.85		0.83	

[a] Models adjusted for gender
[b] Models adjusted for gender and pay type

Table 10. Comparison of the Mutagenic Profiles of Chloroprene, Butadiene and Isoprene

Chemical	In Vitro Ames	In Vivo (B6C3F1 mouse)[a]		
		CA	SCE	Micronuclei
Butadiene	+	+	+	+
Chloroprene	+/-	-	-	-
Isoprene[b]	-	-	+	+

[a] Exposure was 10-12 days (6 hr/day) inhalation (Tice, 1998)
[b] 2-methyl-1,2,3,4-diepoxybutane metabolite is positive (Gervasi and Longo, 1990)

Table 11. Ames Test Results for Chloroprene with TA1535 and/or TA100

Study	Method	Exposure	Response	
			With S9 mix	Without S9 mix
Bartsch *et al*., 1979	Desiccator[a]	4 hours	++	+
Westphal *et al*., 1994	Pre-inc[b]	2 hours	-	-
NTP, 1998	Pre-inc[b]	20 min.	-	-
Willems, 1978; 1980	Desiccator[a]	24-48 hours	++	+

[a]Plates sealed in desiccator at 37° C with tops removed.
[b]Chemical added to sealed tubes and mixed at 37° C.

Table 12. Proto-oncogene Mutation Finger Prints

Chemical	H-*ras*	K-*ras*	Lung	K-*ras*	Lung
	A to T Forestomach	A to T Harderian Gland		G to C Forestomach	
Isoprene	+	+	+	+	-
Chloroprene	+	+	+	-	-
1,3-Butadiene	+	+	-	+	+

Table 13. Exposure-Dose-Response for Rodent Lung Tumors

Species	Exposure concentration (ppm)	PBTK internal dose[a]	Lung tumor incidence	Number of animals	Extra risk incidence (%)[b]
Hamster[c]					
	0	0	0	100	0
	10	0.18	0	97	0
	50	0.88	0	97	0
Wistar rat[c]					
	0	0	0	97	0
	10	0.18	0	13	0
	50	0.89	0	100	0
Fischer rat[d]					
	0	0	3	50	0
	12.8	0.22	3	50	0.3
	32	0.55	6	49	7.7
	80	1.37	9	50	14.0
B6C3F1 mouse[d]					
	0	0	15	50	0
	12.8	3.46	32	50	48.3
	32	5.30	40	50	70.4
	80	7.18	46	50	89.9

[a] Internal dose - average daily mg Chloroprene metabolized/g lung tissue (AMPLU).
[b] See text of Himmelstein *et al*. 2004b for explanation of extra risk calculation.
[c] Male Hamster and Wistar rat data from Trochimowicz *et al.* (1998).
[d] Male Fischer rat and B6C3F1 mouse data from Melnick *et al.* (1999).

Table 14. Study Protocol for *In Vitro* Rate Constants for Metabolism in Liver, Lung and Kidney Microsomes

Task	Species	Sex	Tissue	Endpoints
Prepare microsomes & measure metabolism	Mouse	Male	Kidney	Protein concentration, Total P450, Chloroprene concentration, time course by GC/μECD
		Female	Liver, lung, kidney	
	Rat	Male	Kidney	
		Female	Liver, lung, kidney	
	Human	Pooled	Kidney	
Describe *in vitro* model	(Himmelstein *et al*. 2004)			Documentation of model code
Conduct parameter point estimation	(by ASCL Optimize) [a]			Vmax, Km, & Vmax/Km
Conduct probability analysis	(by Markov Chain Monte Carlo analysis)			Geometric mean (GM) & standard deviation

[a] Included re-analysis of B6C3F1 mouse, F344 rat, & human chloroprene microsomal oxidation data for male liver & lung microsomes from Himmelstein *et al*. (2004).

FIGURES

Figure 1. Respiratory Cancer RRs and SMRs by Cumulative Chloroprene Exposure, Louisville

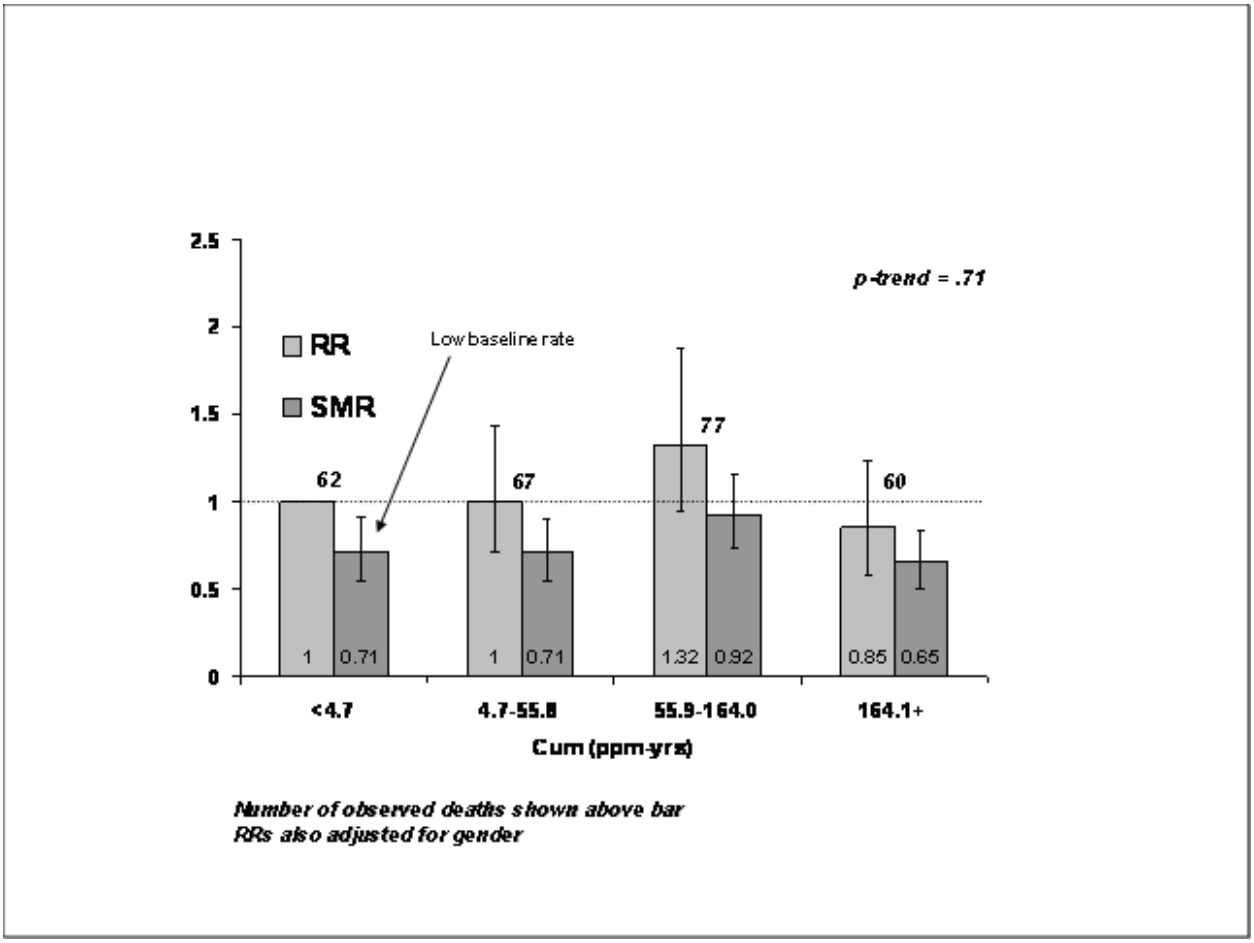


Figure 2. Respiratory Cancer RRs and SMRs by Cumulative Chloroprene Exposure, Grenoble

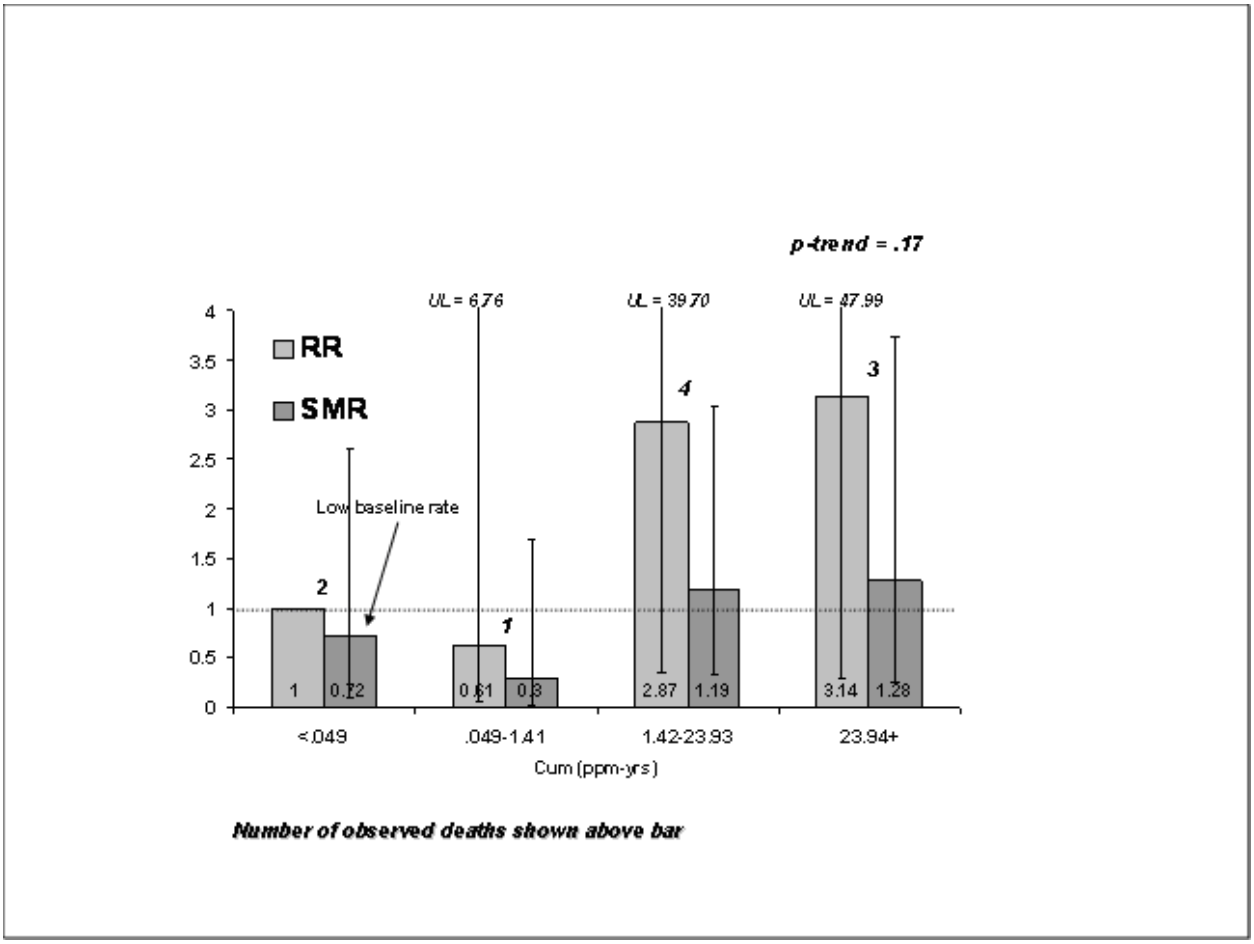


Figure 3. Respiratory Cancer RRs and SMRs by Cumulative Chloroprene Exposure, Maydown

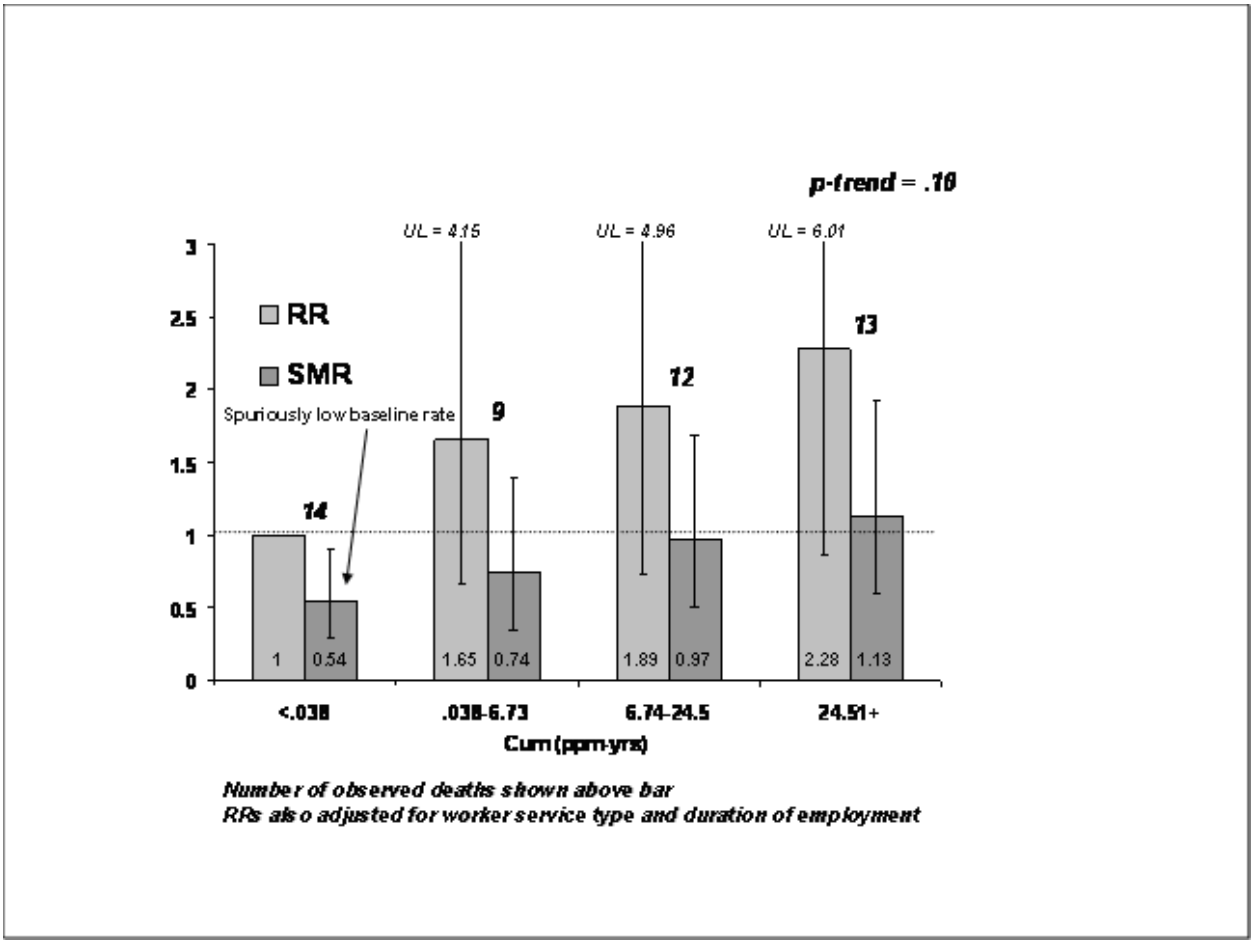


Figure 4. Respiratory Cancer RRs and SMRs by Cumulative Chloroprene Exposure, Pontchartrain

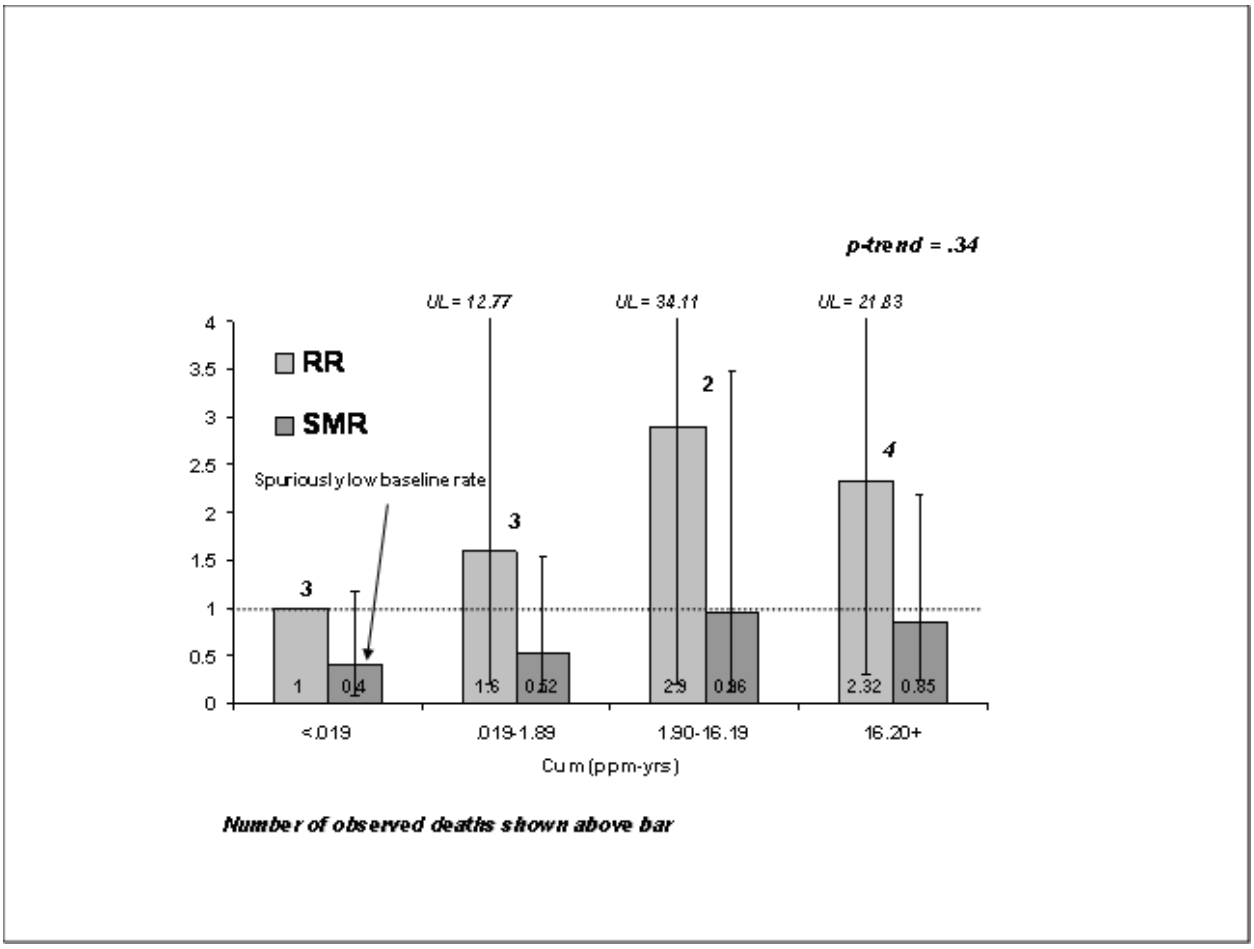


Figure 5. Liver Cancer RRs and SMRs by Cumulative Chloroprene Exposure, Louisville

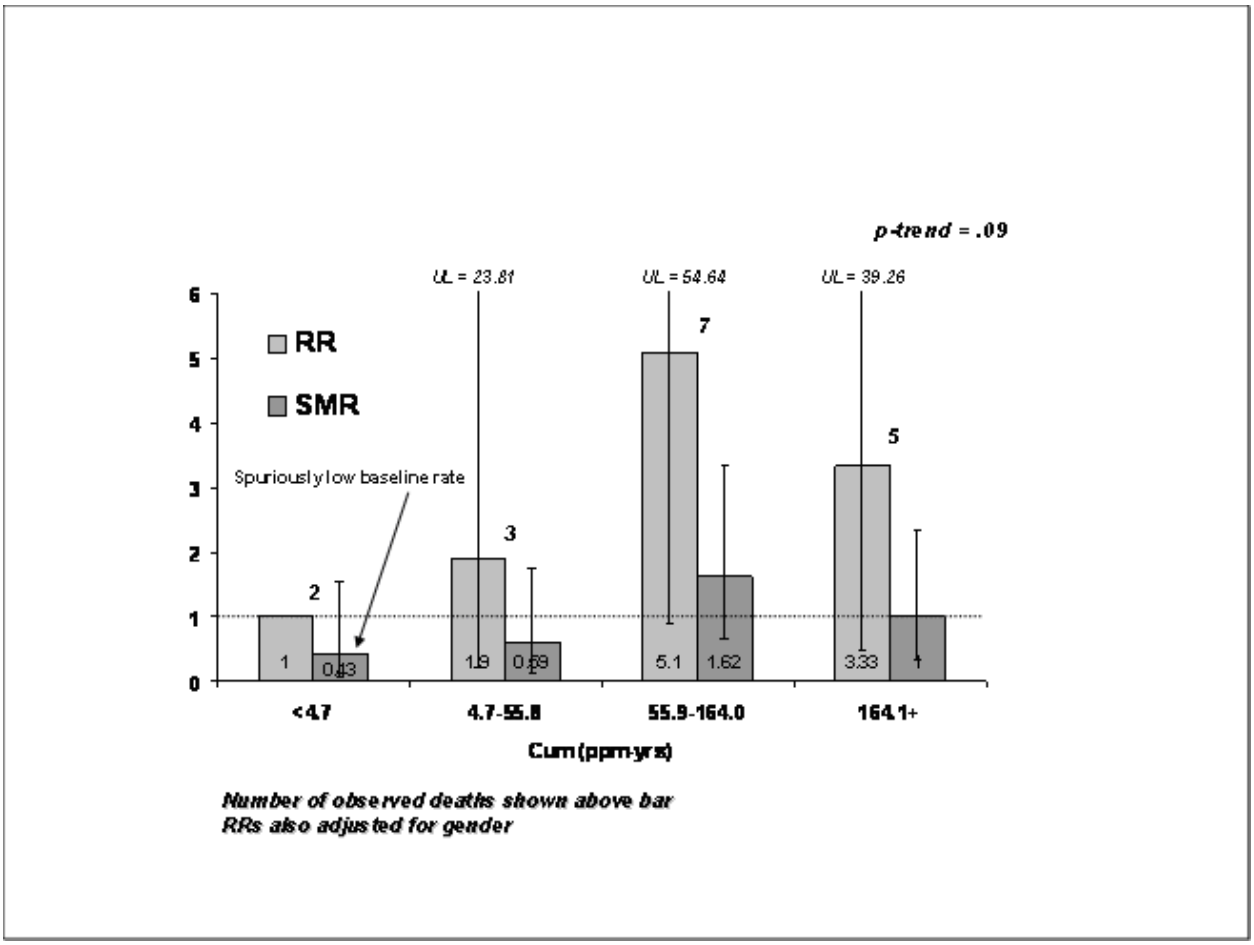


Figure 6. Liver Cancer SMRs by Cumulative Chloroprene Exposure, Louisville

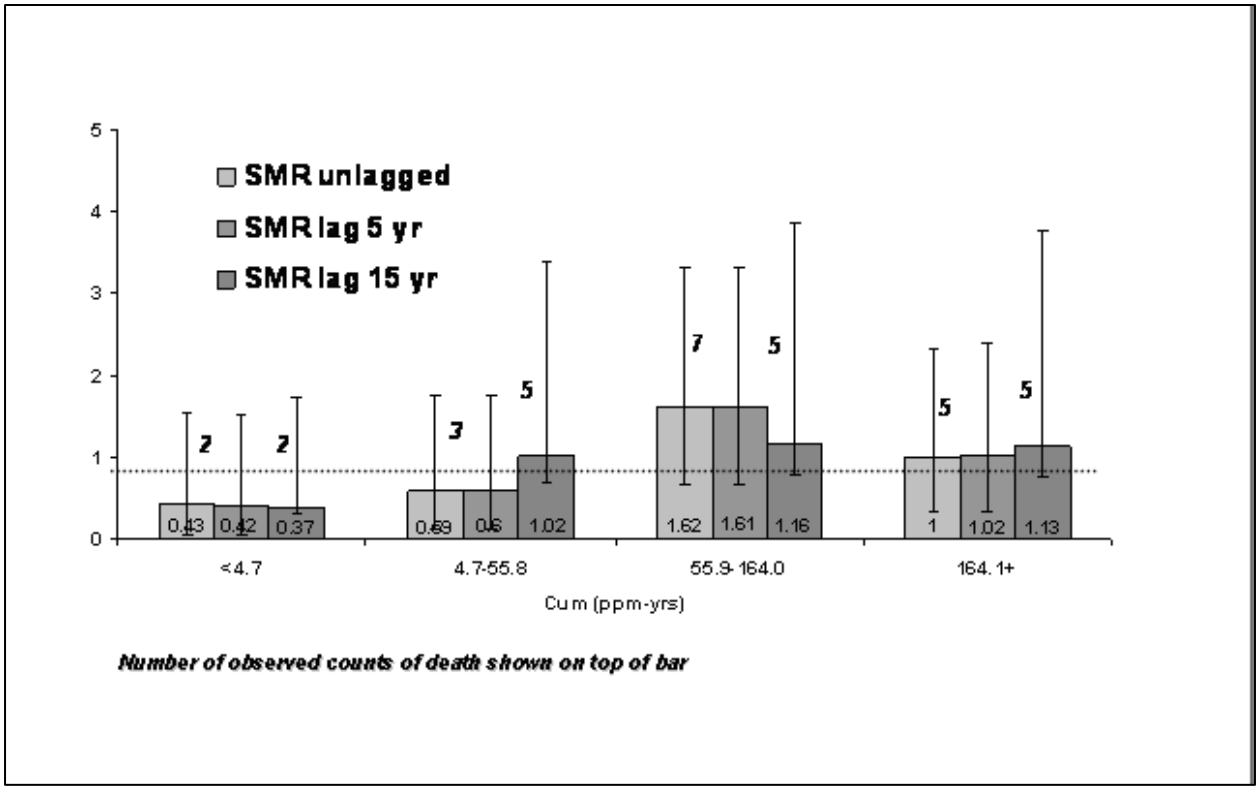


Figure 7. Scattergrams of Chloroprene versus Vinyl Chloride Exposure at Louisville and Maydown

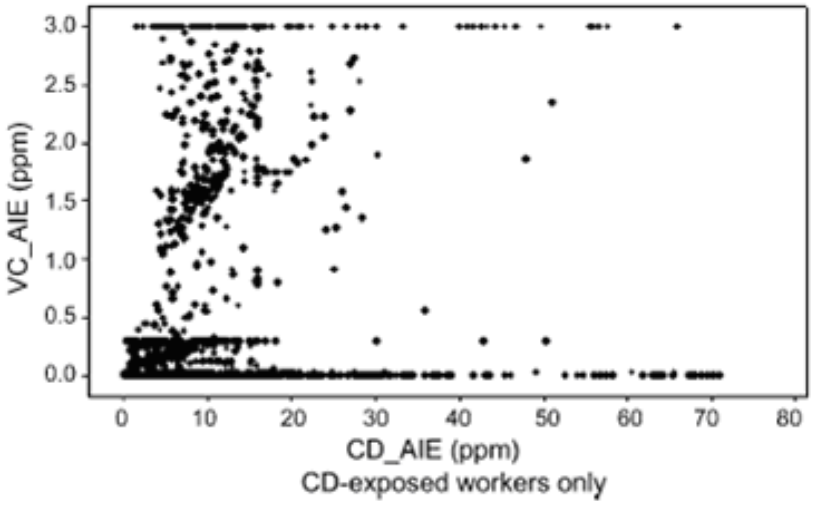


Fig. 7a Louisville plant, VC_AIE by CD_AIE.

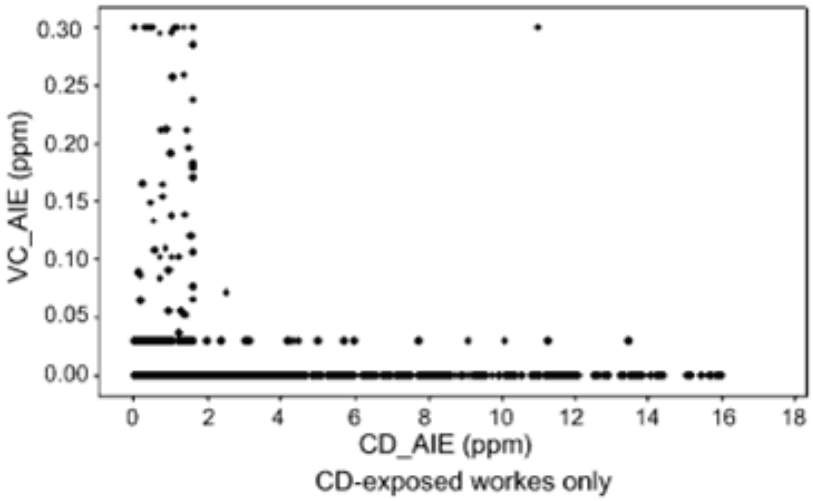
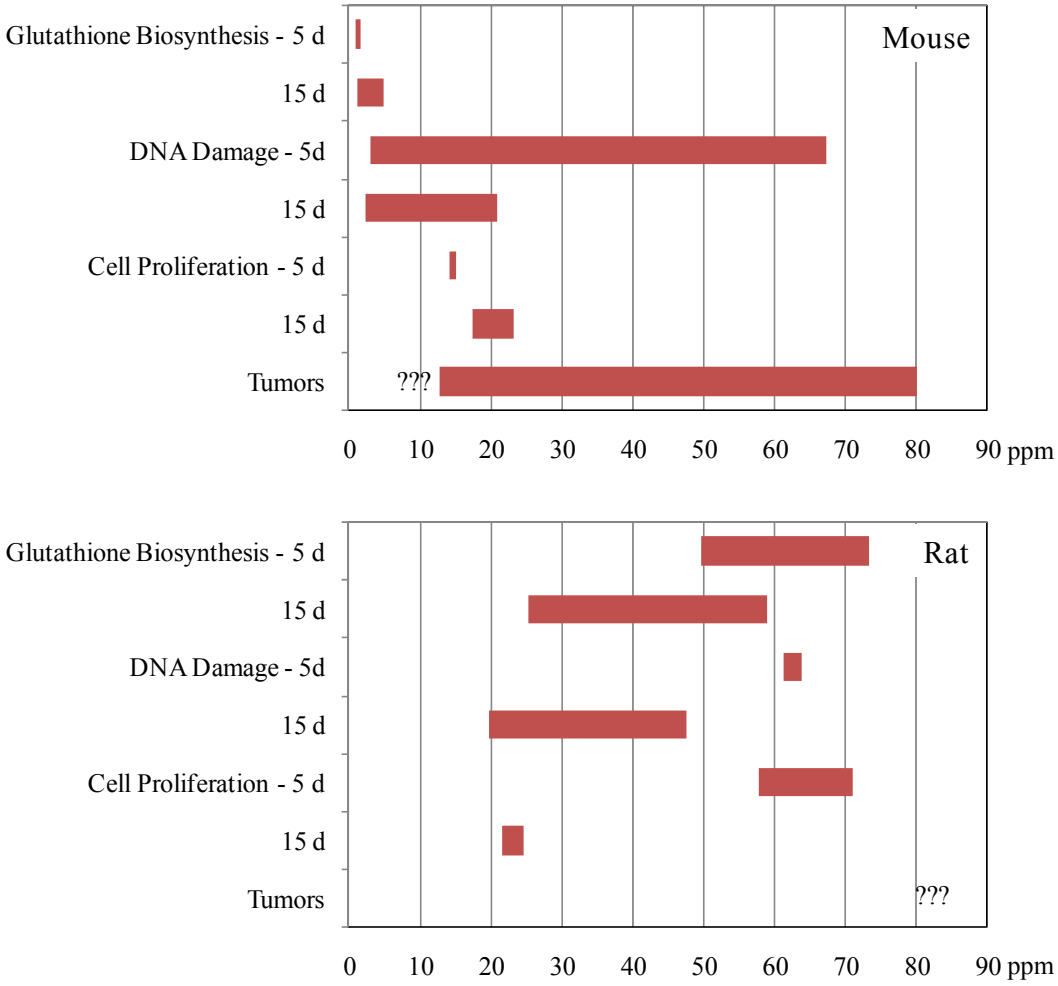


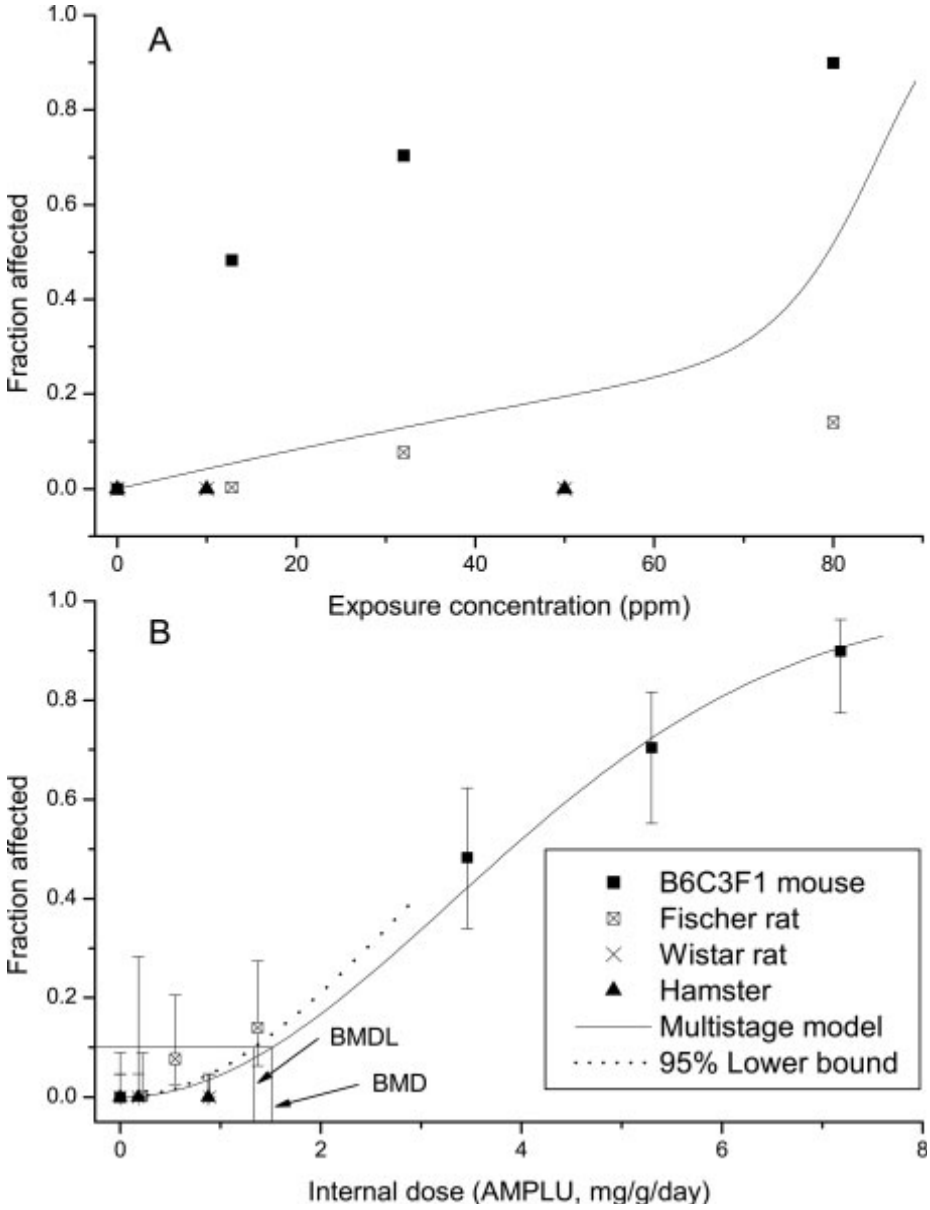
Fig 7b Maydown plant, VC_AIE by CD_AIE.

Figure 8. Gene Expression BMD Values Grouped by Gene Ontology (GO) Category and KEGG (Kyoto Encyclopedia of Genes and Genomes) Pathway.



The responses are overlaid based on exposure concentration. In the figure, "???" indicates no NOEL for lung tumors in female mice at 12.8 ppm (top panel) and no significant increase in lung tumors in female rats up to 80 ppm exposure (bottom panel) in the NTP inhalation bioassay.

Figure 9. Multistage Benchmark Dose (BMD_{10}) Model with 95% Confidence Level ($BMDL_{10}$) for Fraction of Animals Affected by Lung Tumors.



Fraction affected as extra risk compared with external exposure concentration (panel A) or internal dose of chloroprene metabolism (panel B) in the lung. Panel B includes curves for BMD and BMD 95% lower bound and model derived confidence intervals for the respective extra risk values. The BMD model *p*-value was 0.0107; only the BMD curve is given in panel A due to poor fit (BMD *p*-value = 0.0000). The fraction affected which was corrected for extra risk and the derivation of internal dose is described in Himmelstein *et al* 2004b.

Figure 10. Effects of Chloroprene Exposure on Survival in Male and Female Rat and Mice (NTP, 1998)

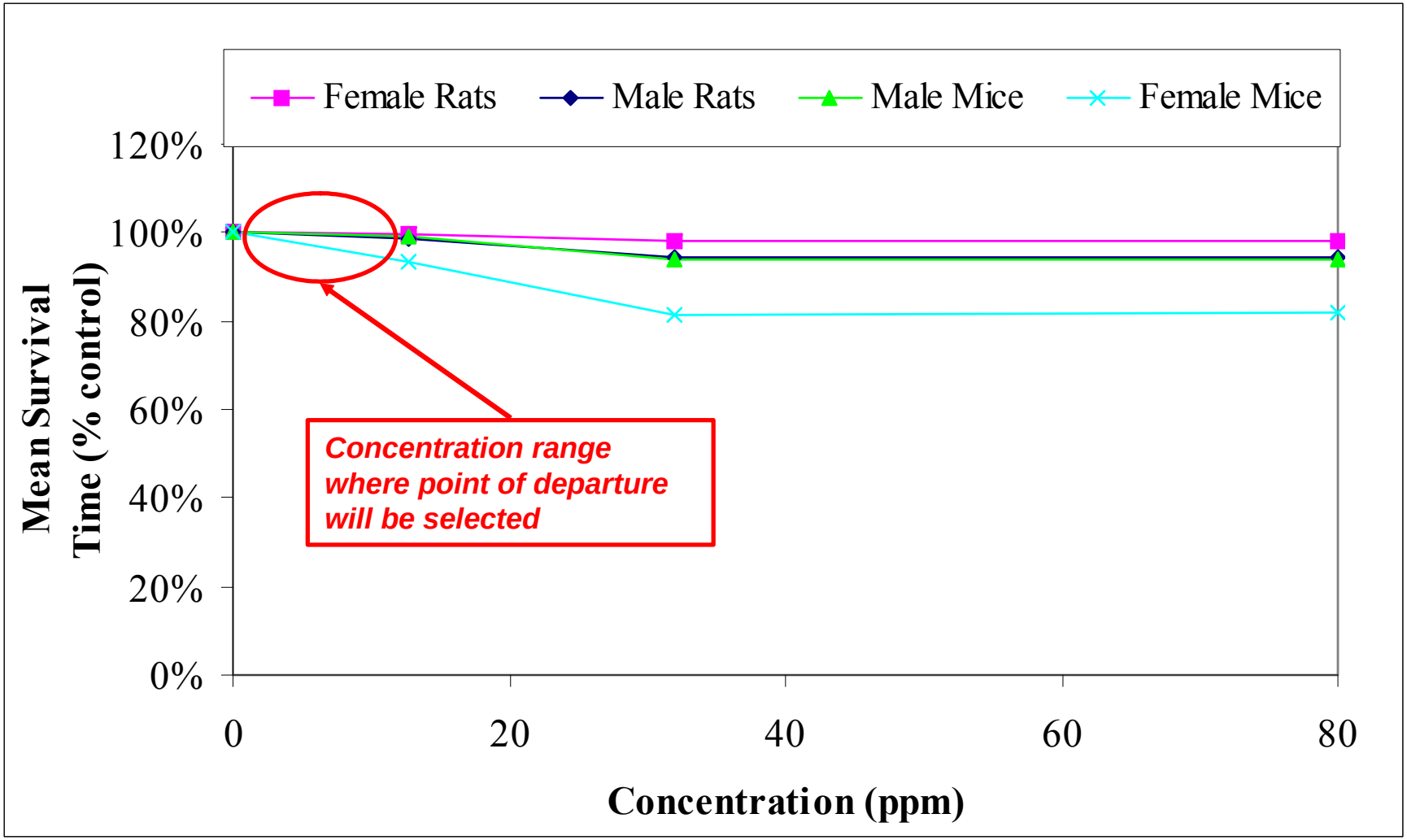


Figure 11. Comparison of Concentration-Response Data for Lung and Circulatory Tumors in Female Mice Exposed to Chloroprene (NTP, 1998)

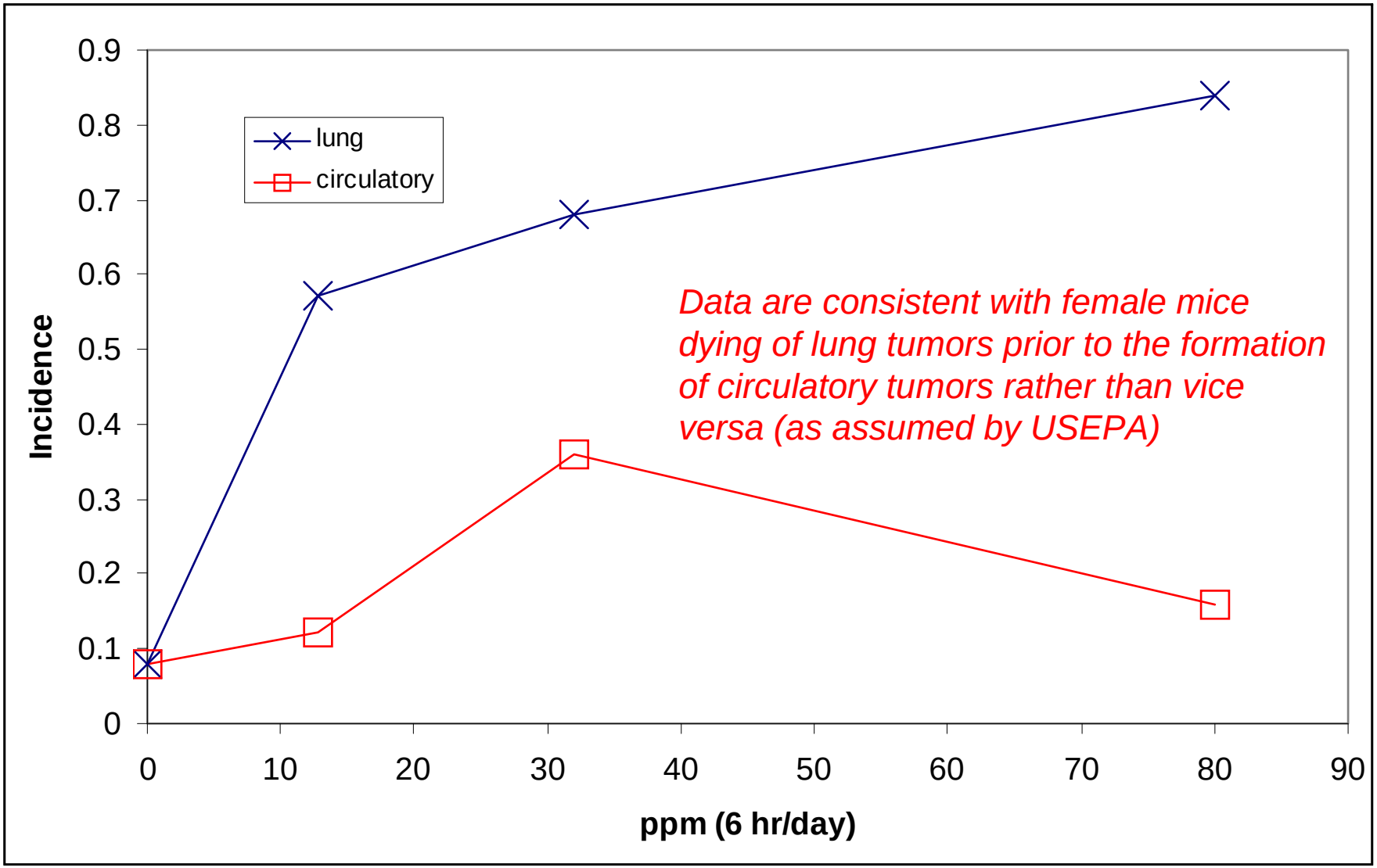
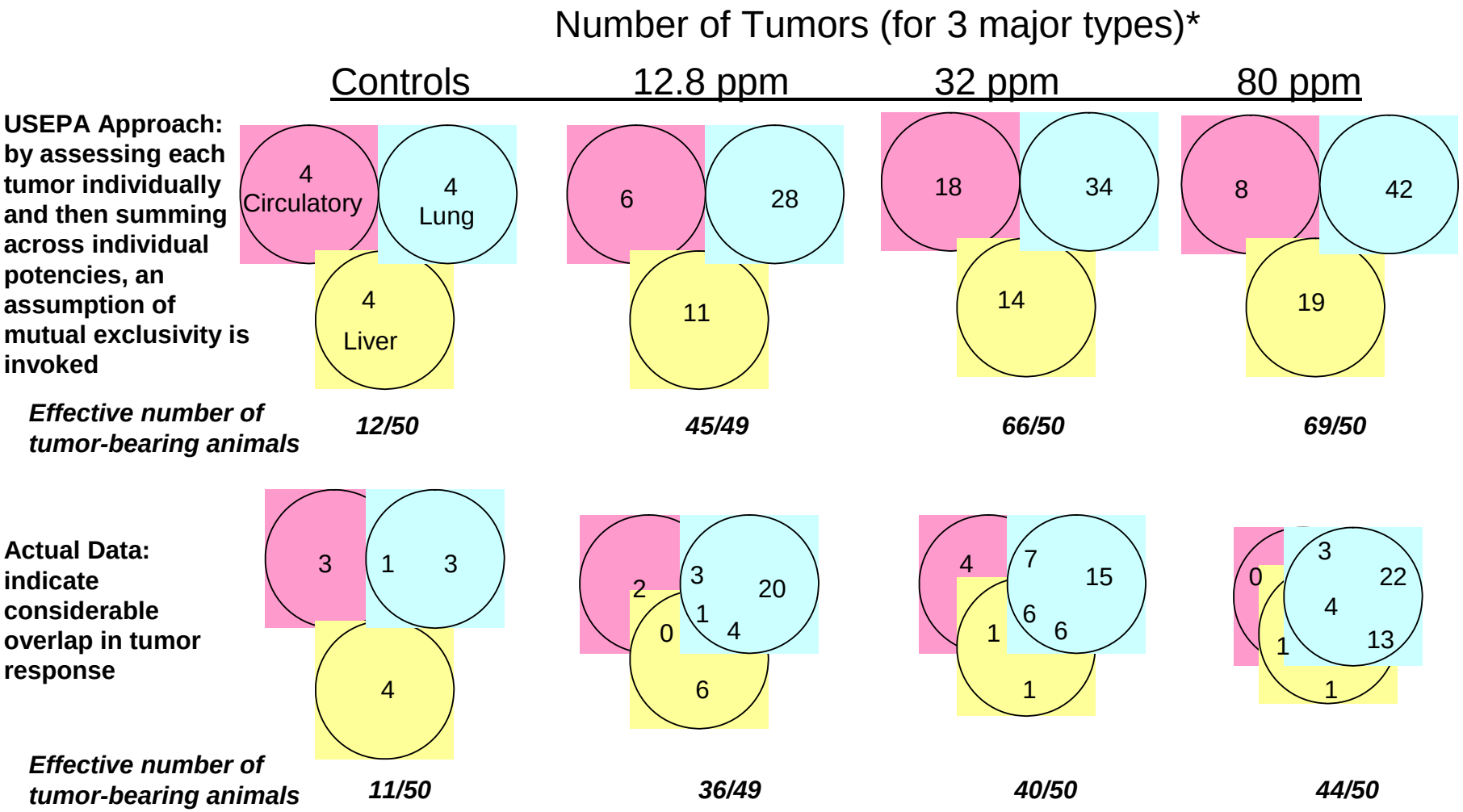


Figure 12. Illustration of How USEPA's Approach to Summing Individual Tumor Potencies Overestimates Total Potency in Female Mice



*Inclusion additional tumor types used by USEPA (total of 8) would make these figures too complicated to present, and therefore only the 3 major tumor types are presented here for illustrative purposes

Attachment A - Critique of Available Epidemiology Data

Below is a more detailed discussion provided by the investigators of the Marsh *et al*. (2007a, b) studies for four of the epidemiological studies considered in the Draft Review. Each discussion includes a brief description of the study, the strengths and weaknesses of each study and comments on the how the results have been interpreted in the Draft Review. Application of the ten quality criteria for evaluating an epidemiological study as reported by Bukowski (2009) and outlined in the Guidelines for Carcinogen Risk Assessment (USEPA 2005) is also discussed. Table A1 provides a summary of the methodological strengths and weaknesses of each study.

Bulbulyan *et al*. (1998)

Description of the study

Bulbulyan *et al*. (1998) summarizes the results of a historical cohort study of shoe factory workers in Moscow, Russia that was conducted to assess the relationship between exposure to chloroprene (from glue and polychloroprene latex) and the risk of cancer. The study included 5,185 (4569 women, 616 men) shoe manufacturing production workers who were employed at least two years in 1960-1976. The study period was from 1979-1993 during which the cohort contributed 70,328 person-years in total. Detailed work histories were available for all workers but no chloroprene exposure assessment was performed. Chloroprene exposure was classified into three groups (no exposure, medium exposure and high exposure) using the detailed work histories and industrial hygiene data from the 1970-79 time period. Numeric values of 0, 1, and 10 were assigned to the exposure groups (no exposure, medium, high) to enable estimation of cumulative exposure to chloroprene. Standardized mortality ratios were computed using Moscow population based rates. Internal comparisons were also performed using Poisson regression modeling.

Overall mortality was close to expected (SMR=1.03, 95% CI=0.97-1.10) while overall cancer mortality was statistically significantly elevated (SMR=1.22, 95%CI=1.07-1.37). There was an over 2-fold statistically significant increase in liver cancer based on ten cases (SMR=2.4, 95%CI=1.1-4.3) and a slight increase in lung cancer risk that was not statistically significant (n=31, SMR=1.4, 95%CI=0.9-2.0). There were consistent increases in liver cancer by chloroprene exposure levels but none were statistically significant (RRs=1.0, 3.8, 4.9). There was a statistically significant trend of increasing levels of risk for liver cancer across increasing levels of duration of chloroprene exposure (p=.02). However, this was based on only three cases (one each level of exposure) which led to imprecise estimates as shown by the very wide confidence intervals (RRs=1.0, 2.7, 8.3, 45.0). No trend in liver cancer risk across levels of cumulative exposure was noted, however, RRs were elevated in the two highest levels of exposure (RR=7.1, 95%CI=0.8-61.0; RR=4.4, 95%CI=0.4-44.0). There was a not statistically significant slight increase in lung cancer risk for the highest chloroprene exposure level, but no trend was evident across all three levels (RRs=1.0, 0.9, 1.1). Lung cancer RRs were elevated across levels of duration of exposure to chloroprene for the 1-9 years and 10-19 years groups (RRs=1.3, 2.0). There were no lung cancer deaths in the 20+ years of exposure category. No trend in lung cancer risk across levels of cumulative exposure was noted.

The authors conclude that based on their study results chloroprene exposure increases the risk of liver cancer.

Strengths and Limitations

One of the limitations of Bulbulyan *et al*. (1998) was the incomplete follow-up and death ascertainment of the cohort. While workers hired between 1960 and 1976 with at least two years of employment were included in the cohort, study follow-up was only conducted from 1979-1993 due to the unavailability of the mortality data. Workers who died before 1979 were then excluded. This most likely led to an underestimate of the SMR. It is not clear if workers who terminated before 1979 were also excluded. Another limitation that most likely affected the SMR for liver cancer was that the local county rate used in the SMR was based on a two year period (1992-93). This two year rate was applied to the total time period. Rates based on a two year period are inherently unstable as shown by the extremely wide confidence intervals of the liver cancer SMR.

There was also no exposure assessment performed and exposure computations were based on job title and industrial hygiene data from 1970-79. Qualitative exposure groups were created and assigned a unit of exposure. The assignment of this unit appears to be based on the 1970 IH data and was very subjective. In addition, another limitation was the lack of data on known co-exposures. Other potential confounders for the outcomes of interest were also not accounted for in the analysis.

More troubling was the multivariate Poisson regression modeling performed for liver cancer based on four cases. Exact modeling was not used. Statistically significant trends were shown with extremely elevated RRs however, these were only based on one death and the confidence intervals were very wide. It is not clear how these models were fit based on four cases as they were also adjusted for gender, age and calendar period.

This study has very low statistical power especially for the causes of interest (liver cancer and lung cancer). There were only ten liver cancer deaths of which nine were exposed (three at the highest level). There were only 31 lung cancer deaths of which 23 were exposed (five at the highest level).

The glue used in production at this facility is made from polychloroprene in a solvent, not from chloroprene monomer. No information is provided on the "residual" monomer and its relative concentration to the solvent used. In addition, the solvent used is not identified. The assignment to exposure categories of 0, 1, and 10 leads one to assume that there was no separation between consideration of polychloroprene and chloroprene adhesive exposure. Levels of residual monomer only could not lead to orders of magnitude differences in exposure.

Comments on the Consideration of Bulbulyan et al. (1998) in the Draft Review

Bulbulyan *et al*. (1998) conclude that their study suggests that chloroprene exposure increases the risk of liver cancer. They note that even in light of the low statistical power, these conclusions hold and add the evidence of chloroprene carcinogenicity. The Draft Review includes the conclusions as stated by the authors as evidence for the carcinogenic effects of chloroprene in the liver. The review does acknowledge that there were limitations to the study and do not acknowledge that their conclusions are hindered by these limitations.

Despite the severe limitations noted here, section 4.7.2.1.1 of the Draft review uses this study as evidence for temporality, strength of association, consistency and specificity. However, the EPA interpretation can be challenged for several reasons. Temporality is questionable given the fact that Bulbulyan *et al*. (1998) did not consider latency analyses as stated in the Draft Review. Strength of association is also suspect given the fact that the SMRs were computed using a two year regional rate and a small number of observed cases. Both of these limitations led to very imprecise estimates. Consistency and specificity are also questionable given the liver cancer findings were all based on ten liver cancer cases. The internal modeling was based on four cases (one unexposed and three in the high exposure group).

Of the ten criteria used in USEPA (2005) to assess study quality, the Bulbulyan *et al*. (1998) study received a high score on only one (clear objectives), (Bukowski, 2009). The sample size criterion was considered high-medium quality. Four criteria were deemed of medium strength: case ascertainment, control of bias, adequate response and documentation of results. Three of the criteria were considered medium-low strength (comparison groups, follow-up, and data collection and evaluation) and the exposure criterion was considered as low quality.

Bukowski (2009) gave the Bulbulyan *et al*. (1998) study an overall rank of 5 (with 1 being the highest and 6 the lowest), behind all four of the Marsh *et al*. (2007a, b) cohorts and equivalent to the Colonna and Laydevant (2001) study and Bulbulyan *et al*. (1999).

Bulbulyan *et al*. (1999)

Description of the Study

Bulbulyan *et al*. (1999) summarizes the results of a historical cohort study of chloroprene production plant workers in Yerevan, Armenia that was conducted to assess the relationship between exposure to chloroprene and the risk of cancer. The study included 2,314 (1897 men, 417 women) who were employed in production departments at the plant for at least two months between 1940 and 1988. The study period was from 1979-1988 for mortality (21,107 person-years) and from 1979-1990 for incidence (25,782 person-years). Detailed work histories were available for all workers but no chloroprene exposure assessment was performed. Chloroprene exposure was classified based on department and for two time periods (< 1980 and 1980+). Numeric values were assigned to each department/time period group to enable estimation of cumulative exposure to chloroprene. Standardized incidence ratios (SIRs) and Standardized mortality ratios (SMRs) were computed using Armenian national population based rates for incidence and mortality respectively. Internal comparisons were also performed using Poisson regression modeling.

Overall cancer incidence was reduced based on 37 cases of cancer (SIR=0.68, 95% CI=0.49-0.94). The SIR for lung cancer was also reduced based on 6 cases (SIR=0.53, 95%CI=0.24-1.19). There was an over 3-fold statistically significant increase in liver cancer incidence based on six cases (SIR=3.27, 95%CI=1.47-7.27). Except for the short term workers (<1 year of employment, no deaths), there were consistent increases in liver cancer by duration of employment but only the 20+ years category was statistically significant (SIRs=3.69, 2.91, 3.45 (1.29-9.20)). There was evidence of increasing levels of risk for liver cancer across increasing levels of duration of chloroprene exposure, however no deaths occurred during the less than one year group (SIRs=1.90, 4.56). When limited to only those in the high exposed categories, a trend

in liver cancer risk across levels of duration of high exposure was noted (SIRs=1.46, 2.00, 6.12) although the lowest two categories are based on only one case each. Results for cumulative exposure were similar; however, no deaths occurred in the lowest cumulative exposure category. (SIRs=2.93. 4.86). Results of the internal analysis were not shown but it is stated that they were similar to the SIR analysis.

Overall cancer mortality was slightly reduced compared to the total Armenian population (n=20, SMR=0.87, 95%CI=0.56-1.36). Mortality from lung cancer was also reduced based on three deaths (SMR=0.50, 95%CI=0.16-1.55). Similar to the incidence, there was an over 3-fold increase of mortality due to liver cancer that was statistically significant (SMR=3.39, 95%CI=1.09-10.51), however this was only based on three deaths.

The authors conclude that even in the presence of several limitations, the results of this study confirm previous reports that chloroprene exposure might increase the risk of liver cancer.

Strengths and Limitations

One of the limitations of Bulbulyan *et al*. (1999) was the lack of complete follow-up and case and death ascertainment of the cohort. While workers hired between 1940 and 1988 with at least two months of employment were included in the cohort, study follow-up was only conducted from 1979-1990 for incidence and 1979-1988 for mortality due to the unavailability of the data. Workers who were diagnosed before 1979 (for incidence) and died before 1979 (for mortality) were then excluded. This most likely led to underestimation of the SIR and SMR. It is not clear if workers who terminated before 1979 were also excluded. It is also briefly mentioned that there may be under-ascertainment of the liver cancer cases due to incomplete registration and misclassification of the cases in the Armenian national cancer registry.

No exposure assessment was performed and exposure computations were based on production department and two time periods. Each department and time period specific category was assigned a unit of exposure. The assignment of this unit appears to be based very subjective. In addition, data on known co-exposures were not collected so were not included in the analysis. Also not included in the analysis were other potential confounders for the outcomes of interest.

As with Bulbulyan *et al*., (1998), the multivariate Poisson regression modeling was performed for liver cancer based on six cases however, exact modeling was not used. Statistically significant extremely elevated RRs were noted however, these were only based on five highly exposed cases and the confidence intervals were very wide. It is also not clear how these models were fit based on six cases as they were also adjusted for gender, age and calendar period.

This study has very low statistical power especially for the causes of interest (liver cancer and lung cancer). There were only six liver cancer incident cases of all were exposed (five at the highest level). There were only six lung cancer cases.

Comments on the Consideration of Bulbulyan et al. (1999) in the Draft Review

Bulbulyan *et al*. (1999) conclude that despite the limitations of short follow-up and lack of detailed exposure information, their study confirms previous study results suggesting that exposure to chloroprene increases the risk of liver cancer in humans. The Draft Review includes

the conclusions as stated by the authors as evidence for the carcinogenic effects of chloroprene in the liver.

Despite the severe limitations noted above, section 4.7.2.1.1 of the Draft Review uses this study as evidence of strength of association, consistency, and specificity of findings all based on the increased statistically significant liver cancer SIRs and RRs. This study is also cited in the Draft Review as evidence for biological gradient based on trends seen by duration of high exposure for liver cancer incidence. Strength of association, consistency and specificity are suspect given the fact that the SIRs were based on only six cases. The internal modeling based on these six cases was used as evidence for biological gradient however there were no unexposed cases and only one case each in the low and intermediate exposure levels.

Of the ten criteria used in USEPA (2005) to assess study quality, the study received a high score on only one (clear objectives). Five criteria were deemed of medium strength: comparison groups, exposure, case ascertainment, data collection and evaluation, and adequate response. Four of the criteria were considered medium-low strength (follow-up, control of bias, sample size and documentation of results).

Bukowski (2009) gave the Bulbulyan *et al*. (1999) study an overall rank of 5 (with 1 being the highest and 6 the lowest), behind all four of the Marsh *et al*. (2007a, b) cohorts and equivalent to the Colonna and Laydevant study and Bulbulyan *et al*. (1998).

Colonna and Laydevant (2001)

Description of the Study

The Colonna and Laydevant (2001) study appears to be a cancer incidence study of the cohort examined by Romazini *et al*. Cancer incidence was examined from 1979-1997 among 533 male workers exposed to chloroprene for at least 2 years between 1966 and 1997 (n=7,950 person-years). SIRs were calculated using the local Isere region as the reference population. Exposure was assigned qualitatively (1 (low), 2 (medium), 3 (high)) to jobs using atmospheric measurements. Exposure was also examined by duration and by time period (pre- and post-1977 when industrial controls were implemented).

The authors found an overall, not statistically significant, excess of cancer (n=34, SIR=1.26, CI=.88-1.77) with specific excesses in lung cancer (n=9, SIR=1.84, CI=.84-3.49) and head and neck cancer (n=9, SIR=1.89, CI=.87-3.59). Only one case of liver cancer was identified in the cohort. Some statistically significant SIRs were found in the pre-1977 group but as person-years in the post-1977 group are small (only 30% were hired in this period) and employees were younger than those in the pre-1977 group, no conclusions can be drawn from these data (per the authors).

No statistically significant SIRs were found. The highest lung cancer SIR was found in the low exposure group, with risk decreasing as exposure increased. Seven of the nine lung cancer cases were known smokers, one did not have data on smoking available and one was not a smoker.

The authors conclude that their results did not confirm the excess risk of liver cancer found in

other cohorts of chloroprene workers and that the excesses found in other cancers were not compatible with occupational exposures.

Strengths and Limitations

The cancer incidence tracking appears to be thorough and complete. The authors appropriately note the issues with their study, including: no quantitative exposure assessment, small cohort size and low number of person-years and lack of information on co-exposures and potential confounders.

Comments on the Consideration of Colonna and Laydevant (2001) in the Draft Review

In Table 4-7 of the Draft Review, the total cohort and pre-1977 SIRs for selected cancers (only those with elevations) are reported. It is unclear why the pre-1977 comparison was chosen rather than the high exposure group. Colonna and Laydevant (2001) dismiss the pre/post-1977 comparison due to low numbers and the young age of the post-1977 group. A better indicator of an association with chloroprene exposure would be to examine the SIRs in the high exposure group (Table 4 in the paper), as shown below:

Cancer type	**Total cohort Cases, SIR (95%CI)**	**High exposure Cases, SIR (95%CI)**
All cancers	32, 1.26 (.88-1.77)	12, .88 (.46-1.54)
Head and neck	9, 1.89 (.87-3.59)	3, 1.19 (.25-3.48)
Larynx	3, 2.43 (.5-7.13)	2, 3.21 (.39-11.65)
Lung	9, 1.84 (.84-3.49)	3, 1.23 (.26-3.61)
Liver	1, 1.36 (.04-7.63)	0
Colon/rectum	2, .66 (.08-2.39)	0

Section 4.7.2.1.1 of the Draft Review cites Colonna and Laydevant (2001) as evidence of temporality with liver cancer based on the latency results. This is impossible for two reasons. The authors identified only one case of liver cancer in their cohort, which cannot be used as evidence of an association. Also, the authors do not specifically examine latency. They consider duration of exposure and the period of time in which exposure first occurred; they do not consider the length of time between first exposure and development of disease. The authors themselves conclude that there is no evidence of an association between chloroprene and cancer.

Bukowski (2009) included the Colonna and Laydevant study in his evaluation of the epidemiologic evidence for chloroprene carcinogenicity. Of the 10 criteria used by USEPA to assess study quality, the study received a high-medium score on only one (clear objectives). Seven criteria were deemed of medium strength: comparison groups, exposure, case ascertainment, control of bias, data collection and evaluation, adequate response and documentation of results. Follow-up was of medium to low quality and sample size was low.

Bukowski gave the Colonna and Laydevant study an overall rank of 5 (with 1 being the highest and 6 the lowest), behind all four of the Marsh *et al*. (2007a, b) cohorts and equivalent to the Armenian (Bulbulyan *et al*., 1999) and Russian (Bulbulyan *et al*., 1998) studies.

Li *et al.* (1989)

Description of the Study

This manuscript presents the results of a cohort and case-control study of workers exposed to chloroprene. Cancer deaths were ascertained from July 1, 1969 to June 30, 1983. Fifty-five cancer deaths were identified in the cohort; 16 of these had exposure to chloroprene. The authors matched 54 of the cancer deaths to noncancer deaths in a case-control study. They found a total cancer SMR among the chloroprene workers (n=16) of 2.38 (stat sig). They stated that the total cancer SMRs were higher among the chloroprene workers with high exposure jobs compared to workers with low exposure jobs, indicating a dose-response relationship. They also investigated cancer-specific SMRs in subgroups and found that cancer deaths among maintenance mechanics (n=4), a high exposure group, occurred for liver (n=2), lung (n=1) and lymphoma (n=1) cancers; they attributed these deaths to the carcinogenic effects of chloroprene.

The authors conclude that chloroprene exposure increases the risk of developing cancer.

Strengths and Limitations

This study has no discernible strengths. The many methodological flaws are discussed in detail below.

The entrance criterion for the study was stated as more than one year of work in a chloroprene-exposed job before June 30, 1980, yet on pg 142 the authors state "The occupations of the 1258 persons selected…included only those that could be classified according to the levels of exposure". It is unclear what this means in terms of work history abstraction. Were non-chloroprene jobs not captured? It is also unclear where the cancer deaths in non-chloroprene workers came from (Table 2) or how people were grouped into occupational categories. Finally, there is never a mention of the number of person-years associated with the cohort.

The facility used the acetylene process throughout its history but no mention is made of exposure to VC, a known liver carcinogen.

Only cancer deaths were included. These deaths were identified through records at the plant's hospital and police substation. This would not be the way in which cancer deaths were identified in the general (comparison) population. Differential identification of cancer deaths is a serious bias and negates the SMR (population-based) comparisons.

The SMR comparison was made using sex and age-specific cancer mortality in the local area from 1973-1975. Such a short time period and small area of comparison make the rates inherently unstable. No valid conclusions can be drawn using such rates.

The authors attribute 16 of the 54 cancer deaths identified to "chloroprene exposure". How was this determined when the entrance criterion was one year of work in a chloroprene-exposed job and only occupations with chloroprene exposure were included?

The authors only provide all cancer SMRs for the 16 chloroprene workers (Table 3). They show an additional 38 cancer deaths in Table 2. What are the SMRs for the non-chloroprene workers?

The authors indicate statistical significance for the SMRs but do not provide confidence intervals.

The expected values for the SMRs are extremely small. Very few of the occupational subgroups examined have an overall cancer expected value greater than 1. The only cancer-specific expected value greater than 1 is for liver cancer in the total cohort. The lung cancer expected value in the total cohort is 0.39. The expected number of liver cancers in the total cohort is 2.48 – more than 6 times the expected number for lung cancers. This raises serious questions about the person-years involved in this study (never shown) and the validity of using a 3-year local area as the comparison population. The pattern of expected values is very unusual. Also, because the expected values are so small if even one cancer occurs, the SMR is extremely high. This is the case for the majority of the SMRs shown.

The exposure assessment is very questionable. Although the authors state that levels of chloroprene in the air were available, they used worker and administrators opinions to classify the jobs into exposure levels. The plant was divided into three units: monomer workshop, neoprene workshop and laboratory; there were 5 high exposure jobs (2 monomer, 2 neoprene, 1 lab) and 3 low exposure jobs (1 monomer, 1 neoprene, 1 laboratory) identified (Table 1). Researcher was identified as a low exposure job.

Although person-years are never noted, this study is presumed to have very low statistical power to detect meaningful differences in lung or liver cancer SMRs. The expected number of liver cancers was 2 and the expected number of lung cancers was less than 1.

In the summary statements, the authors state that "SMRs for all cancers in all high-exposure occupations were of significance". This is not correct. Of the 5 high exposure jobs, only 3 were statistically significant (monomer operator, n=4, SMR=4.50; maintenance mechanic - monomer, n=4, SMR=12.9; polymer operator, n=5, SMR=3.94); the other two were not statistically significant (maintenance mechanic – neoprene, n=0; quality monitor, n=1, SMR=1.29). The authors also state that "the SMRs in the low-exposure occupations were not significant". This is also not correct. One of the three low-exposure occupational categories had a very high statistically significant SMR (researcher, n=2, SMR=11.76); the other two low-exposure jobs had no cases. The authors go on to state, "Thus, a dose-response relationship was present". The summation of these inaccurate statements does not supply evidence of a dose-response relationship, especially given that no quantitative exposure assessment was done nor were any analyses conducted relating duration of exposure to the outcome.

Comments on the Consideration of Li et al. (1989) in the Draft Review

The Draft Review includes the conclusions as stated by Li *et al*. as evidence for the carcinogenic effects of chloroprene. The Draft Review does indicate that there were some limitations to the study including using three years of local area data as the mortality comparison, having no quantitative exposure information, being unable to examine the findings by latency or duration of employment and having limited data on co-exposures or potential confounders (pages 4-5). The Draft Review does not note the many other troublesome limitations as listed above, including: the very small expected values in the SMR calculations, the inconsistencies in the cohort entrance criteria and population shown in the manuscript, the inconsistent way in which

cancer deaths were ascertained in the study and general populations, the inaccurate characterization of exposure levels shown in the text and tables, the lack of data on non-chloroprene exposed workers although those deaths appear in Table 2, the incorrect summary statements.

In Section 4.7.2.1.1 (Evidence for Causality), the Draft Review cites the Li *et al*. (1989) study multiple times as evidence for cancer causality in humans without regard to any of the severe limitations of the study. Li *et al*. (1989) is cited as evidence of strength of association, based on the statistically significant SMRs for liver cancer. The Draft Review does not mention the serious problems with the cancer ascertainment in the study nor the limitations of their local comparison and very small expected values.

The elevated liver SMRs reported by Li *et al*. (1989) are also cited as examples of consistency and specificity of findings – again with no mention of the serious methodological limitations of the study.

Bukowski (2009) utilized ten criteria used by the USEPA to assess the quality of each of the studies used to assess chloroprene carcinogenicity. Li *et al*. did not receive a high rating for any of the ten; they received high-medium for two criteria, medium for one, medium-low for three and low for three. Bukowski (2009) gave the Li *et al*. (1989) study the lowest possible overall rank of 6.

Summary

Outside of the Marsh *et al*. (2007a, b) study, the evidence of the carcinogenicity of chloroprene in the Draft Review is based on four studies discussed above: Bulbulyan *et al*. (1998;1999), Colonna and Laydevant (2001) and Li *et al*. (1989). These studies have serious methodological limitations that were not given proper consideration in the Draft Review.

Table A1 shows the major methodological strengths and weaknesses of the Marsh *et al*. (2007a, b) and other studies (Bulbulyan *et al*., 1998, 1999; Colonna & Laydevant, 2001; Li *et al*., 1989). These studies have fewer strengths and many more methodological weaknesses than the Marsh *et al*. (2007a, b) study. As noted in Table A1, only the Marsh *et al*. (2007a, b) cohort had quantitatively estimated historical exposures to chloroprene and was the only study to conduct detailed exposure-response modeling. Li *et al*. (1989) was the only study to note an association between chloroprene exposure and lung cancer, based on only 2 cases. The two Bulbulyan (1998, 1999) and the Li study found associations with liver cancer and chloroprene based on ten or fewer cases in each cohort.

Table A1. Methodological Features of the Marsh *et al.* (2007a,b) and Other Cohort Studies of Chloroprene Workers

Methodological Feature	Marsh *et al.* (2007a, b)				Other Cohorts			
	US - Louisville[1]	US - Pontchartrain	Ireland - Maydown	France - Grenoble	Armenia (Bulbulyan)	France (Colonna & Laydevant)	Russia (Bulbulyan)	China (Li)
Study Population and Follow-up								
Cohort enumeration verified to be complete via formal process	●	●	●	●	○	●	○	○
Inclusion of male and female subjects	●	●	●	●	●	○	●	unk
Availability of information of race/ethnicity of individual subjects	●	●	●	●	unk	●	unk	unk
Inclusion of substantial portion of subjects employed 10+ years and followed 20+ years	●	●	●	●	unk	unk	unk	unk
Vital status found for >95% of cohort	●	●	●	●	●	n/a	●	●
Cause of death found for >95% of known deaths	●	●	○	○	unk	n/a	unk	unk
Exposure Assessment								
Availability of detailed work histories for all individual members of the cohort	●	●	●	●	●	●	●	unk
Quantitatively estimated historical exposures to chloroprene	●	●	●	●	○	○	○	○
Qualitatively estimated historical exposures to vinyl chloride	●	●	●	●	○	○	n/a	○
Qualitatively estimated historical exposures to co-exposures known or suspected to be carcinogens	○	○	○	○	○	○	○	○
Cancer incidence study with cases identified by tracing cohort through national, regional or hospital tumor registries	○	○	○	○	●	●	○	○

Table A-1.
Methodological Features of the Marsh *et al.* (2007a, b) and Other Cohort Studies of Chloroprene Workers (continued)

Methodological Feature	Marsh *et al.* (2007a, b)				Other Cohorts			
	US - Louisville[1]	US - Pontchartrain	Ireland - Maydown	France - Grenoble	Armenia (Bulbulyan)	France (Colonna & Laydevant)	Russia (Bulbulyan)	China (Li)
Statistical Analysis								
80% or greater statistical power to detect 2.0-fold or greater excess in lung cancer among subjects exposed to chloroprene	●	●	●	●	○	○		○
80% or greater statistical power to detect 2.0-fold or greater excess in liver cancer among subjects exposed to chloroprene	●	○	○	○	○	○	○	○
External mortality comparisons via SMRs based on national rates	●	●	●	●	●	n/a	○	○
External mortality comparisons via SMRs based on local rates	●	●	○	○	○	n/a	●	●
Internal mortality comparisons via relative risk regression modeling or Poisson regression analysis of internal cohort rates	●	●	●	●	●	○	●	○
Detailed exposure-response modeling of quantitative measures of chloroprene exposure and lung cancer	●	●	●	●	○	○	○	○
Detailed exposure-response modeling of quantitative measures of chloroprene exposure and liver cancer	●	n/a	n/a	n/a	○	○	○	○

Table A-1.
Methodological Features of the Marsh *et al.* (2007a, b) and Other Cohort Studies of Chloroprene Workers (continued)

Methodological Feature	Marsh *et al.* (2007a, b)				Other Cohorts			
	US - Louisville[1]	US - Pontchartrain	Ireland - Maydown	France - Grenoble	Armenia (Bulbulyan)	France (Colonna & Laydevant)	Russia (Bulbulyan)	China (Li)
Authors' conclusions regarding carcinogenicity of chloroprene								
Respiratory system/Lung cancer	No association, based on 266 cases	No association, based on 12 cases	No association, based on 48 cases	No association, based on 10 cases	No association, based on 6 mortality and 3 incident cases	No association, based on 9 cases	No association, based on 31 cases	Association, based on 2 cases
Liver cancer	No association, based on 17 cases	No cases identified	No association, based on 1 case	No association, based on 1 case	Association, based on 6 mortality and 3 incident cases	No association, based on 1 case	Association, based on 10 cases	Association , based on 6 cases

[1] The Louisville cohort included in the Marsh *et al*., (2007) study is the same as that examined by Pell (1978) and updated by Leet and Selevan (1982). Because the Marsh update has the most complete follow-up period, only those data are considered.

● Feature present
○ Feature absent
n/a Feature not applicable to this study
unk. Information needed to evaluate feature not available in paper

Attachment B - Toxicokinetic Study in Mice

In a recently completed toxicokinetic study (IISRP-12828-1388, 2009), female B6C3F1 mice were exposed to chloroprene via inhalation either one day (nose-only) or repeatedly (whole body) for up to 5 or 15 days. In the single exposure experiment, mice were exposed to chloroprene at concentrations of 0, 13, 32, or 90 ppm chloroprene with whole blood samples collected at 0, 0.5, 3, and 6 hours during exposure and 5, 10, or 15 minutes following the cessation of exposure after 6 hours (5 mice per exposure concentration per time-point). In addition, plethysmography measurements (minute volume) were collected during the 6-hour exposure (4 mice/exposure concentration). In the repeat exposures experiments, mice were exposed at the same chloroprene concentrations listed above over the course of 5 or 15 days (5 days a week for 1 or 3 weeks, 5 mice per exposure concentration per time-point). In this repeated exposure study, chloroprene concentrations in blood were measured prior to the start of exposure and after 6 hours of exposure on days 5 and 15.

After a single exposure, chloroprene in whole blood increased rapidly by the 0.5-hour time point, with maximum chloroprene concentrations occurring at the 3- or 6-hour time points. As demonstrated in Figure B-1, increasing the exposure concentration resulted in a nearly proportional increase in blood concentrations after a single (6-hour) exposure (maximum concentrations less than 8 μM). Upon cessation of a single exposure, blood chloroprene concentrations declined rapidly to non-detectable levels by 15 minutes after the end of the 6-hour exposure period in the 13 ppm exposure group and to less than 1 μM in the 32 and 90 ppm exposure groups (a reduction of approximately 90%).

With repeated exposure for 4 days, no chloroprene was detected in blood just prior to the start of day 5 exposure, indicating essentially complete clearance of chloroprene overnight between exposures (subsequent measurements prior to day 15 were not made). At hour 6 of the 5th or 15th day of exposure, maximum blood concentrations were 2 to 9 fold lower than those measured at hour 6 after a single exposure (Figure B-2).

Possible explanations for the decreased blood chloroprene concentrations observed in the repeat exposure group relative to the single exposure included reduction of the minute volume and/or an increase in chloroprene metabolism with repeated exposure. Increased metabolism by induction of cytochrome P450 was tested in a companion study in mice (IISRP-12828-1406, 2009) which has also been submitted to Docket ID No. EPA-HQ-ORD-2009-0217 on December 1, 2009. The results of this companion study showed no increase in total P450 or several individual P450 isozymes. A likely scenario is that ventilation rates were higher during the single 6-hour exposure because of stress, while repeated exposures resulted in adaptation and lower ventilation rates.

Figure B-1. Chloroprene in Blood of Female Mice During & After 6-Hour Nose-Only Exposure

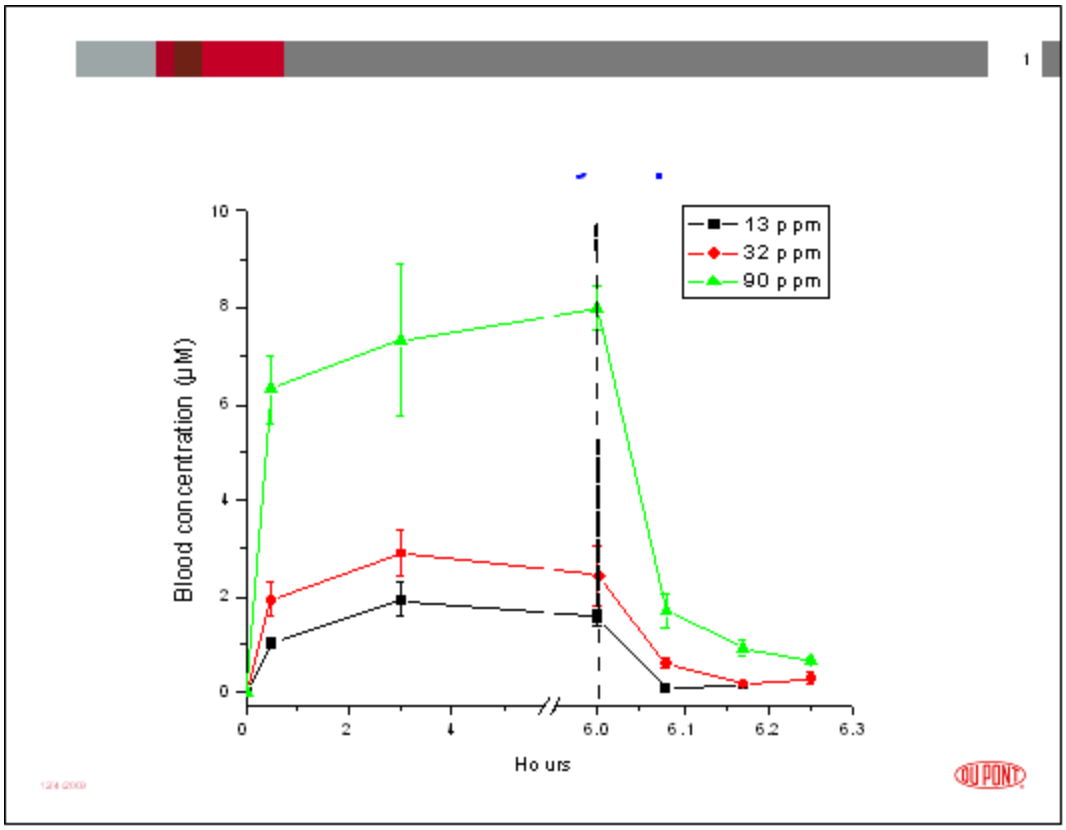
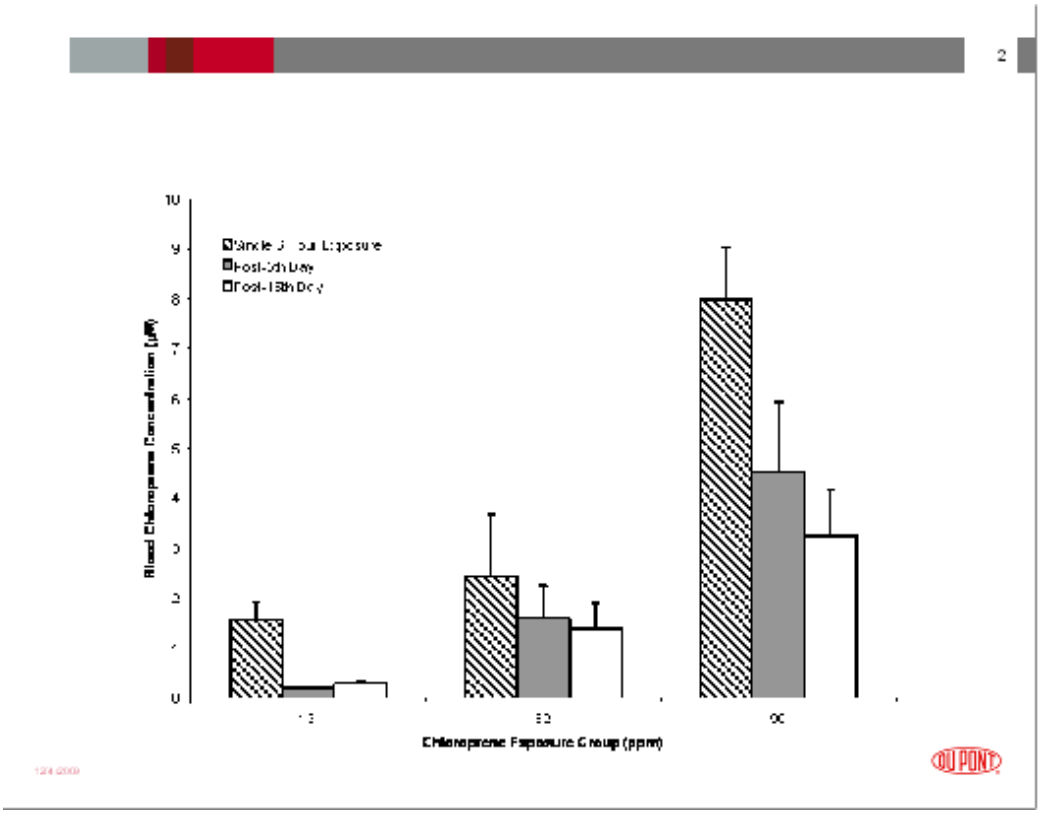


Figure B-2 – Single and Repeated Exposure 6-Hour Blood Concentration of Chloroprene in Mice



Attachment C - Section Specific Comments

Chapter 2: Physical/Chemical Properties

- **Page 2-1, line 1 Chloroprene is listed as a flammable liquid in the Draft Review, but should be referred to as a reactive monomer.** There is some mixing in the literature of information on chloroprene the monomer and chloroprene, the synthetic rubber, more properly polychloroprene. The USEPA's 1989 Health and Environmental Effects Document for Chloroprene is a good resource for information on the chemical/physical properties of chloroprene. It is suggested that the referenced page and line in the Draft Review be revised to state: "β-chloroprene monomer (C4H5Cl) (hereafter referred to as chloroprene) is a volatile, flammable liquid used in the manufacture of polychloroprene or neoprene rubber."
- **Page 2-1, line 3 Neoprene is listed as being "used to make diverse products, such as tires, wire coatings…."** Tires are not made from Neoprene rubber although numerous automotive components do use polychloroprene rubber. General applications consistent with polychloroprene use are shown. It is suggested that the reference page and line in the Draft Review be revised to read: "Polychloroprene rubber is used to make diverse products, such as adhesives, automotive or industrial parts (e.g., belts/hoses/gaskets), coatings, and dipped goods."
- **Page 2-1, line 4 The Draft Review states "neoprene solid, polychloroprene" and "polychloroprene latex".** The polychloroprene is the same polymer in both references. The term latex is often confused with natural rubber latex and associated allergenicity. Suggested revisions: "…solid polychloroprene (trade names include neoprene, Bayprene, Skyprene, Butaclor, etc.) and polychloroprene liquid dispersions.
- **Page 2-1, lines 6-8 It states in the Draft Review that, "In 1995, there was one commercial producer …"** This is outdated information; there were two producers, one in Houston (ex-Bayer facility) the other Ponchartrain (DuPont). The Houston facility closed shortly after 1995. Since this is obsolete information suggested revisions to the text include: "In 2008, there was one commercial producer in the United States; this site both manufactured the monomer and converted it to polymer. Chloroprene is used almost exclusively to produce polychloroprene, with chloroprene monomer sold to only one U.S. company for non-polychloroprene manufacture (1000 lbs in 2008)."
- **Page 2-1, lines 6-8 In the Draft Review, it is noted that chloroprene is a volatile, flammable liquid**. However, there is no information on how chloroprene is generated, an omission important to understanding the epidemiology. A description of the different manufacturing processes (one butadiene based the other acetylene based) is needed with the implications for co-exposure to mono/di vinyl acetylene and/or vinyl chloride. Suggested revisions include adding text from the 2001 IISRP symposium (from Lynch 2001) at line 22 to include: "Commercial manufacture of chloroprene involves either acetylene or butadiene feedstocks. Chloroprene manufacture using butadiene as starting material occurs via a two step process consisting of chlorination and subsequent dehydrochlorination reactions. Initial industrial processes (1930's – 1970's) for chloroprene manufacture involved the dimerization of acetylene and then its

hydrochlorination to produce chloroprene monomer. The butadiene process to polychloroprene can result in volatile emissions consisting of mixed chloroprene dimers, peroxides, and other oxidized chloroprene species. In contrast, the acetylene process yields monochloro/dichloro-vinylacetylene and vinyl chloride reaction byproducts."

- **Page 2-1, lines 9-10 The Draft Review states that chloroprene is sold to only three US companies.** This is outdated with the plant closures noted above. Consider following text revision or deletion, since suggested revisions were made for lines 6-8 (above): "In 2008, chloroprene monomer was sold to only one external company for non-polychloroprene manufacture(1000 lbs)."
- **Page 2-1, line 12 The Draft Review states that "The volume from 1995 to 1996 was approximately 200 to 250 million lb (90,700 to 113,000 metric tons)."** This misrepresents the current use of chloroprene which has been decreasing and is lower now than the reference time frame. DuPont Performance Elastomers is the only domestic producer and production of chloroprene monomer in 2008 was less than 40,000 metric tons. Suggested revisions to the text include: "The volume from 1995 to 1996 was approximately 200 to 250 million lb (90,700 to 113,000 metric tons). Chloroprene manufacture has decreased since 1996; in 2008, US production volume was below 40,000 metric tons."
- **Page 2-1, lines 15-16 The Draft Review states that, "Main sources of emissions are from …or transport of the product."** With the plant closures listed above, no bulk chloroprene has been shipped since 2008. The phrase "or transport of the product" should be deleted since bulk shipments of chloroprene no longer occur.
- **Page 2-1, lines18-21 The Draft Review states that "Three plants in Kentucky, Texas ….converted chloroprene to polychloroprene (NTP, 2005)."** However, this text no longer reflects current status of chloroprene facilities, because the Texas and Kentucky plants have been permanently shut down. Suggested revisions to the text include: "In 2008, only one chloroprene plant remained open (as noted above) with reported releases (LA DEQ) of 210,900 lbs. Domestic production and releases have been decreasing (reported 2002 emissions were 356,700 lbs)."
- **Page 2-2, line 8 The Draft Review states that "Spontaneously….. form dimers and other oxygenated species unless inhibitors are added."** However, inhibitors will not prevent the Diels-Alder dimerization reaction. It can be slowed by storage at low temperatures. Suggested revisions to the text include: "…spontaneously dimerizes and oxidizes forming peroxides and other oxygenated species. Stabilizers/inhibitors must be added to prevent peroxide formation and consequent spontaneous polymerization; inhibitors do not reduce dimer formation."
- **Page 2-2, lines 13-14 The Draft Review states that, "Reaction products may account for observed toxicity and may be questionable unless storage/ vapor generation conditions are reported."** This is an important observation and should be considered when selecting studies for citation or as the basis for Inhalation UR. This statement is especially relevant if the test atmospheres are produced by heating chloroprene monomer. The text should be revised to emphasize this observation.
- **Page 2-2, lines 20-21 The potential fate of chloroprene that is released to soil is to leach into groundwater.** Chloroprene has the potential to leach to groundwater if released into soil; however, rapid volatilization into air will mitigate downward movement in soil. Suggested revisions would be to add statement: "The potential fate of

chloroprene that is released to soil is to leach into groundwater; however, rapid volatilization into air will mitigate downward movement in soil."

- Page **2-2, lines 21 Breakdown by hydrolysis is not likely.** Simply stating low solubility does not mitigate hydrolysis. No guideline chloroprene hydrolysis studies are available in the literature and substances deemed structurally similar have markedly different hydrolysis rates. Hughes *et al*., (2008) investigated the dechlorination of chloroprene in simulated groundwater amended with iron, including a pH 6-7 DI water control at 10C. The recovery appears to be 85% after 300 hrs. In contrast, the analog substance, 2,3-dichloro-1,3-butadiene is rapidly hydrolyzed (t ½ = 1 hr at 50C) as shown in OECD SIDS dossier for 1653-19-6.
- **Page 2-2, lines 23-24 (Kow = 2.2)** Bioaccumulation values are based in part, on octanol water partitioning, expressed as **log** Kow. Revise value to read…"(log Kow = 2.2)"
- **Page 2-2, lines 24- 25 The occupational exposure potential to chloroprene is limited to facilities in the U.S., Europe, and Asia.** List is not complete for the current global use of chloroprene. Suggest revision to read: "As of 2008, occupational exposure potential to chloroprene in the US is limited to one site in Louisiana; other chloroprene manufacturing facilities exist in Germany, France, Armenia/Azerbaijan, India, China, and Japan."
- **Page 2-3 Table of Physical/chemical properties** - Not all values are current. Note that alpha-chloroprene is incorrectly listed as a synonym for β-chloroprene. There is no registered trade name for chloroprene. For reasons cited above, chloroprene is a monomer, neoprene is a generic name for polychloroprene rubber. IRIS authors should consult the 1999 OECD SIDS document on chloroprene for additional phys/chem property data. The table should distinguish between modeled and measured values. If modeled, should make clear whether the values obtained are within the models' domain. In addition, when reported measured and calculated values are shown, the origin of each should be clearly denoted within the table, text. Himmelstein *et al*, 2001 measured a liquid to air partition coefficient value, Kp, representing partitioning between a liquid (inactivated microsomal preparation) and the air (vial headspace). A Kp of 0.69 was reported, confirming the compartmentalization into air.

Chapter 4: Chloroprene exposure and non cancer effects

- **Page 4-19, line 1 Nystrom was the first to acknowledge that storage conditions would alter toxicity of chloroprene; oxidized forms are more acutely toxic to rats than freshly prepared.** Consider mentioning observation of oxidized byproducts reported in the early reports of animal and human toxicity. Also note that no systematic assessment of the composition or concentration of components in workplace air was made.
- **Page 4-19, line 5 Studies from 1944-1997.** Typo: Should be 1944-1947
- **Page 4-19, line 10 The Draft Review states that Nystrom attributes hair loss from "systemic rather than direct skin exposure (which was carefully controlled)."** Workplace protective measures were not described in sufficient detail to exclude skin exposure as a contributing exposure route. Suggest deleting the phrase "(which was carefully controlled)" as it adds little to the section.

- **Page 4-19 lines 23-33 and Page 4-20 lines 1-6 Study results from human populations are presented in the Draft Review as provided by the Sanotskii (1976) review paper.** As noted on pg 4-20, the studies described in Sanotskii's (1976) review paper, are largely anedoctal and do not have corroborative information on methods/results, etc, to allow determination of study reliability. For all references involving Sanotskii (14 were identified), suggest either a) deleting reference Sanotskii altogether given lack of ability to interpret; or consider adding USEPA conclusions for each entry where Sanotskii is mentioned. USEPA's position on Sanotskii study reliability is mentioned once on pg 4-20 lines 26-30 such that the results/conclusions are suspect based on prior USEPA analysis (which did not allow interpretation of these results with any reliability).
- **Page 4-21, lines 27-30 and Page 4-21, lines 1-3 Sanotskii study quality.** Since the Sanotskii review article represents secondary data, it provides no information concerning test substance purity, test atmosphere generation or analytical methods. It is a questionable reference to cite in the Draft Review. Conclusions regarding the inability to interpret these mixed studies should apply to all studies referenced by Sanotskii in the Draft Review. The text implies that some studies (e.g., the ones in Table 4-12 and elsewhere) represent valid data suitable for hazard assessment. Consider either deleting the Table or moving conclusions related to Sanotskii's review article to the end of Table 4-12 (on pg 4-21, line 4).
- **Page 4-52, line 23 Additional toxicology data not cited in the Draft Review.** An unpublished 26-wk inhalation toxicity study was conducted as part of the Joint Industry Group on Chloroprene in 1978. This study was not referenced in the Draft Review and the final report is being provided for inclusion. This study conducted at exposure concentrations up to 100 ppm in Wistar rats shows some effects on liver weights and kidney function but no histopathologic changes in tissues.
- **Page 4-53, line 9; Page 4-57, Table 4-36; Page 4-60, line 19; Page 4-61, line 13 ;Page 4-62, line 13; Page 4-65, line 26; Page 6-1, line 25; and Page 6-2, line 6 Sanotskii study quality**. See previous recommendations on Sanotskii study quality.

Chapter 4: Individual Occupational Studies

- **Page 4-9, lines 15-24: the text describes the internal analysis when it is actually the SIR analysis**. The SIRs listed are correct; the text incorrectly describes the type of analysis.

- **Page 4-12, lines 20-21: the document indicates that external mortality comparisons were made in the four Marsh *et al.* study cohorts using local county rates.** Marsh *et al*. (2007a, b) used both U.S. and local county rates for the two U.S. cohorts (Louisville and Pontchartrain), but only national rates were used for the Maydown and Grenoble cohorts.

- Page 4-12, line 26: The lower bound of the confidence interval for the SMR should be 53 not 52.

- **Table 4-10: All values for the four Marsh *et al.* study cohorts are footnoted as letter "e" standardized incidence ratios (SIRs).** These are standardized mortality ratios (SMRs).

- **<u>Table 4-11:</u> It is not clear why this table shows only relative risk values for liver cancer for the Marsh *et al.* study cohorts.** Table 4-10 showed only SMRs for lung cancer. The values in Table 4-11 for the Marsh *et al.* study cohorts should be footnoted as c. Relative risk of death from live cancer.

- **Page 4-11, the Draft Review states that "a significant trend was observed when data were analyzed by duration of exposure".** On page 508 of Colonna and Laydevant (2001), Section 3.3 Exposure Duration, the authors state "none of these trends were statistically significant". The Draft Review should be clear when discussing statistical significance.

- **Table 4-7 is mislabeled.** The SIRs shown in the table for Colonna and Laydevant (2001) are not all "elevated" cancer risks as the values in this title indicate. Colon/rectum cancer is not elevated.

- **Table 4-2 which cites data from the Li *et al*. (1989) study is incorrect**. The two cases of liver and one case of lung cancer noted under "neoprene workshop" occur in polymer operators not in maintenance mechanics as the table shows.

Chapter 4: Synthesis of Human, Animal, and Other Supporting Evidence – Human

- **<u>Page 4-68, line 30:</u> The SMR for liver cancer for the Li *et al*. (1989) study is incorrect and the SMRs are in the incorrect order for the four studies listed.** The correct liver cancer SMR for Li *et al*. (1989) is 242. For Bulbulyan *et al*. (1999, 1998), Li *et al*. (1989), and Leet and Selevan (1982), the correct order of liver cancer SMRs is 339, 240, 242, 571.

Chapter 5: Dose-Response Modeling

There were errors in the tables or output associated with the time-to-tumor modeling of the neoplastic lesions in the mice.

1. The modeled results from bioassay values reported for the BMDL and BMD for the female mouse for all organs hemangiosarcomas, hemangiomas in Table 5-7 does not match the data reported in Appendix C. As a result of this mistake, the continuous human equivalents are also wrong, but the Unit Risk reported is correct (but cannot be derived from the BMDL value reported in the table). Some of the Unit Risks reported differ in the last digit from those calculated based on the output given in Appendix C.
2. There are inconsistencies in the reported number of animals in several of the TOX_RISK output listings in Appendix C. Some of the animal count values reported in Appendix C do not match the data in Table 4-27 (female mice lung tumors and Zymbal's gland tumors, and male mice renal tubule tumors). Both the zymbal gland and the Harderian gland were not examined microscopically in all animals. In the female mice only the 3 animals in the 80 ppm group were examined for the zymbal gland, but if the number of animals necropsied was being used to determine the number of animals in each group for that analysis, why are only 49 animals reported for the 32 ppm group in the output in Appendix C of the zymbal gland modeling? In other cases, the number of animals at risk

in a group is 50 and does not exclude the animal that should be excluded due to missing tissue or autolysis. Specifically:

- Female mouse #350 in the 12.8 ppm group apparently was excluded from the liver analysis, but not from the forestomach, skin, mammary gland, or lung analyses – although all these organs were marked with *A: Autolysis precludes examination* for that animal;
- Female mouse #118 in control group - mammary gland marked with *M: Missing tissue*;
- Female mouse #138 in the control group - skin marked *A: Autolysis precludes examination;*
- Male mouse #530 in the 12.8 ppm group - kidney marked with *A: Autolysis precludes examination;*
- Male mice #214 and #245 in 12.8 ppm group and #424 in the 32 ppm group - forestomach marked *A: Autolysis precludes examination.*

3. There was one female mouse in the 80 ppm group accidentally killed on day 3 – animal #738. This animal adds no pertinent information to the analyses but it was included in every female mouse analysis.
4. The incidence of forestomach squamous cell papillomas or carcinomas in the 80 ppm group for male mice is incorrect in Table 4-27 and in Appendix C. According to the NTP TR467, animal #s 628, 649, 633, and 637 have squamous cell papillomas and animal 629 has a squamous cell carcinoma.

We were unable match the output as reported in Appendix C of the Draft Review for chloroprene in several cases where our incidence counts did agree. Since our time data was obtained from the online NTP database and we were using TOX_RISK v 5.3 for our analyses, these differences suggest that the time values put in for some of the animals in the TOX_RISK analysis may be inaccurate. Examples of the input data sets should have been provided so that this information can be checked and creation of a printout of the dataset in a compact format is available in TOX_RISK v5.3.